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Trying 31060000009999...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
 ******** HHHHHHHH SSSSSSSS? ### Status: Signing onto Dialog ********
ENTER PASSWORD:
 ******** HHHHHHHH SSSSSSSS?  ********
Welcome to DIALOG

### Status: Login successfulDialog level 05.17.01D

Last logoff:  09apr07 14:39:46
Logon file405 12apr07 08:56:33
          *** ANNOUNCEMENTS ***
                    ***
NEW FILES RELEASED
***BIOSIS Previews Archive (File 552)
***BIOSIS Previews 1969-2007 (File 525)
***Engineering Index Backfile (File 988)
***Trademarkscan - South Korea (File 655)

RESUMED UPDATING
***File 141, Reader's Guide Abstracts
                    ***
RELOADS COMPLETED
***File 5, BIOSIS Previews - archival data added
***Files 340, 341 & 942, CLAIMS/U.S. Patents - 2006 reload now online
                              ***
DATABASES REMOVED

Chemical Structure Searching now available in Prous Science Drug
Data Report (F452), Prous Science Drugs of the Future (F453),
IMS R&D Focus (F445/955), Pharmaprojects (F128/928), Beilstein
Facts (F390), Derwent Chemistry Resource (F355) and Index Chemicus
(File 302).
                    ***
 >>>For the latest news about Dialog products, services, content<<<
 >>>and events, please visit What's New from Dialog at <<<
 >>>http://www.dialog.com/whatsnew/.  You can find news about<<<
 >>>a specific database by entering HELP NEWS <file number>.<<<
* * *
SYSTEM:HOME
Cost is in DialUnits
Menu System II: D2 version 1.8.0 term=ASCII
                    *** DIALOG HOMEBASE(SM) Main Menu ***

 Information:
  1.  Announcements (new files, reloads, etc.)
  2.  Database, Rates, & Command Descriptions
  3.  Help in Choosing Databases for Your Topic
  4.  Customer Services (telephone assistance, training, seminars, etc.)
  5.  Product Descriptions

 Connections:
  6.  DIALOG(R) Document Delivery
  7.  Data Star(R)
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      /H = Help            /L = Logoff          /NOMENU = Command Mode


Enter an option number to view information or to connect to an online
 service.  Enter a BEGIN command plus a file number to search a database
(e.g., B1 for ERIC).
?
Terminal set to DLINK
                      *** DIALOG HOMEBASE(SM) Main Menu ***

 Information:
  1.  Announcements (new files, reloads, etc.)
  2.  Database, Rates, & Command Descriptions
  3.  Help in Choosing Databases for Your Topic
  4.  Customer Services (telephone assistance, training, seminars, etc.)
  5.  Product Descriptions

 Connections:
  6.  DIALOG(R) Document Delivery
  7.  Data Star(R)

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      /H = Help            /L = Logoff          /NOMENU = Command Mode


Enter an option number to view information or to connect to an online
 service.  Enter a BEGIN command plus a file number to search a database
(e.g., B1 for ERIC).
? b biosci

>>>          44 is unauthorized
>>>          76 is unauthorized
>>>2 of the specified files are not available
       12apr07 08:56:43 User276653 Session D100.1
            $0.00    0.281 DialUnits FileHomeBase
     $0.00  Estimated cost FileHomeBase
     $0.03  TELNET
     $0.03  Estimated cost this search
     $0.03  Estimated total session cost   0.281 DialUnits


SYSTEM:OS  - DIALOG OneSearch
  File   5:Biosis Previews(R) 1926-2007/Apr W1
         (c) 2007 The Thomson Corporation
*File   5: BIOSIS has been enhanced with archival data. Please see
HELP NEWS 5 for information.
  File  24:CSA Life Sciences Abstracts 1966-2007/Dec
         (c) 2007 CSA.
  File  28:Oceanic Abstracts 1966-2007/Dec
         (c) 2007 CSA.
  File  34:SciSearch(R) Cited Ref Sci 1990-2007/Apr W1
         (c) 2007 The Thomson Corp
  File  35:Dissertation Abs Online 1861-2007/Mar
         (c) 2007 ProQuest Info&Learning
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  File  40:Enviroline(R) 1975-2007/Feb
        (c) 2007 Congressional Information Service
  File  41:Pollution Abstracts 1966-2007/Dec
        (c) 2007 CSA.
  File  45:EMCare 2007/Apr W2
        (c) 2007 Elsevier B.V.
  File  50:CAB Abstracts 1972-2007/Mar
        (c) 2007 CAB International
  File  65:Inside Conferences 1993-2007/Apr 11
        (c) 2007 BLDSC all rts. reserv.
  File  71:ELSEVIER BIOBASE 1994-2007/Apr W2
        (c) 2007 Elsevier B.V.
  File  73:EMBASE 1974-2007/Apr 10
        (c) 2007 Elsevier B.V.
  File  91:MANTIS(TM) 1880-2006/Jan
        2001 (c) Action Potential
  File  98:General Sci Abs 1984-2007/Apr
        (c) 2007 The HW Wilson Co.
  File 110:WasteInfo 1974-2002/Jul
        (c)  2002 AEA Techn Env.
*File 110: This file is closed (no updates)
  File 135:NewsRx Weekly Reports 1995-2007/Apr W1
        (c) 2007 NewsRx
  File 136:BioEngineering Abstracts 1966-2007/Dec
        (c) 2007 CSA.
  File 143:Biol. & Agric. Index 1983-2007/Mar
        (c) 2007 The HW Wilson Co
  File 144:Pascal 1973-2007/Apr W1
        (c) 2007 INIST/CNRS
  File 155:MEDLINE(R) 1950-2007/Apr 10
        (c) format only 2007 Dialog
  File 164:Allied & Complementary Medicine 1984-2007/Apr
        (c) 2007 BLHCIS
  File 172:EMBASE Alert 2007/Apr 10
        (c) 2007 Elsevier B.V.
  File 185:Zoological Record Online(R) 1978-2007/Apr
        (c) 2007 The Thomson Corp.
  File 357:Derwent Biotech Res. _1982-2007/Apr W2
        (c) 2007 The Thomson Corp.
  File 369:New Scientist 1994-2007/Dec W1
        (c) 2007 Reed Business Information Ltd.
  File 370:Science 1996-1999/Jul W3
        (c) 1999 AAAS
*File 370: This file is closed (no updates). Use File 47 for more current
information.
  File 391:Beilstein Reactions 2007/Q1
        (c) 2007 Beilstein GmbH
  File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
        (c) 2006 The Thomson Corp
  File 467:ExtraMED(tm) 2000/Dec
        (c) 2001 Informania Ltd.

      Set  Items  Description
      ---  -----  -----------
? s endotoxin
     S1  161876  ENDOTOXIN
? s bacteriophage(n)protein
```

```
Processing
Processed  20 of  29 files ...
Completed processing all files
         158212  BACTERIOPHAGE
        9683224  PROTEIN
      S2    1724  BACTERIOPHAGE(N)PROTEIN
? s s1 and s2
         161876  S1
           1724  S2
      S3       1  S1 AND S2
? t s3/9,k/1

 3/9,K/1     (Item 1 from file: 73)
DIALOG(R)File  73:EMBASE
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12016512     EMBASE No: 2003127822
  Sequence and structural diversity in  endotoxin -binding dodecapeptides
  Zhu Y.; Ho B.; Ding J.L.
  J.L. Ding, Marine Biotechnology Laboratory, Department of Biological
  Sciences, National University of Singapore, 10 Kent Ridge Cresent,
  Singapore 117543  Singapore
  AUTHOR EMAIL: dbsdjl@nus.edu.sg
  Biochimica et Biophysica Acta - Biomembranes ( BIOCHIM. BIOPHYS. ACTA
  BIOMEMBR. ) (Netherlands)   01 APR 2003, 1611/1-2 (234-242)
  CODEN: BBBMB   ISSN: 0005-2736
  DOCUMENT TYPE: Journal ; Article
  LANGUAGE: ENGLISH   SUMMARY LANGUAGE: ENGLISH
  NUMBER OF REFERENCES: 47

  For the study of sequence or structure requirement of short peptides for
endotoxin  binding, and to search for potential  endotoxin  antagonists,
biopanning was carried out on a phage-displayed random dodecapeptide
library against immobilized lipopolysaccharide (LPS) or lipid A (LA), the
core toxic portion of LPS. Specific binding of selected phage-displayed
peptides to LPS/LA was confirmed by surface plasmon resonance (SPR)
analysis. These peptides are rich in basic and hydrophobic amino acids,
especially histidine, proline and tryptophan, highlighting apparent
amphiphilicity and bacterial membrane activity. These dodecapeptide
sequences have no predictable secondary structure in solution, indicating
the importance of a random structure before their interaction with LPS/LA.
Sequence alignment reveals various potential secondary structures with
these selected peptides, which contain specific signature motifs such as
b(p)hb(p)hb(p), bbbb, hhhh (b - basic, p - polar, h - hydrophobic residue),
capable of binding LPS/LA. However, none of these peptides exhibit a
significant calculated structural amphiphilicity while assuming a secondary
structure. This study suggests that for these short dodecapeptides to bind
LPS/LA, the potential for their structural adaptation is more important
than an amphipathic structure. (c) 2003 Elsevier Science B.V. All rights
reserved.

DRUG DESCRIPTORS:
* endotoxin ; *peptide derivative
lipopolysaccharide; tryptophan; unclassified drug
MEDICAL DESCRIPTORS:
protein structure;  bacteriophage ;  protein binding ; surface plasmon
resonance; hydrophobicity; bacterial membrane; amino acid sequence; protein
```

motif; article; priority journal
DRUG TERMS (UNCONTROLLED): dodecapeptide
CAS REGISTRY NO.: 6912-86-3, 73-22-3 (tryptophan)
SECTION HEADINGS:
  029 Clinical and Experimental Biochemistry

  Sequence and structural diversity in endotoxin -binding dodecapeptides

  For the study of sequence or structure requirement of short peptides for endotoxin binding, and to search for potential endotoxin antagonists, biopanning was carried out on a phage-displayed random dodecapeptide library against immobilized lipopolysaccharide...
DRUG DESCRIPTORS:
* endotoxin ; *peptide derivative
MEDICAL DESCRIPTORS:
protein structure; bacteriophage ; protein binding ; surface plasmon resonance; hydrophobicity; bacterial membrane; amino acid sequence; protein motif; article; priority journal
? s bacteriophage and protein
Processing
Processed 20 of 29 files ...
Completed processing all files
          158212 BACTERIOPHAGE
         9683224 PROTEIN
      S4   56941 BACTERIOPHAGE AND PROTEIN
? s s1 and s4
          161876 S1
           56941 S4
      S5   63 S1 AND S4
? s s5 and detect
              63 S5
          805056 DETECT
      S6   2 S5 AND DETECT
? s s5 and detect?
              63 S5
         6789758 DETECT?
      S7   7 S5 AND DETECT?
? s s5 and remov?
              63 S5
         2029770 REMOV?
      S8   9 S5 AND REMOV?
? s s5 and incubat?
              63 S5
         1228212 INCUBAT?
      S9   5 S5 AND INCUBAT?
? t s7/9,k/1-7

 7/9,K/1   (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2007 The Thomson Corp. All rts. reserv.

05790106   Genuine Article#: WX602   Number of References: 20
Title: Delivery of a PCR amplified DNA fragment into cells: A model for
    using synthetic genes for gene therapy
Author(s): Li S; Brisson M; He Y; Huang L (REPRINT)
Corporate Source: UNIV PITTSBURGH,SCH MED, DEPT PHARMACOL, LAB DRUG
    TARGETING, W1351 BIOMED SCI TOWER/PITTSBURGH//PA/15261 (REPRINT); UNIV

PITTSBURGH,SCH MED, DEPT PHARMACOL, LAB DRUG TARGETING/PITTSBURGH//PA/15261
Journal: GENE THERAPY, 1997, V4, N5 (MAY), P449-454
ISSN: 0969-7128 Publication date: 19970500
Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE, HAMPSHIRE, ENGLAND RG21 6XS
Language: English Document Type: ARTICLE
Geographic Location: USA
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: PHARMACOLOGY & PHARMACY; GENETICS & HEREDITY; BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: Synthetic genes offer many potential advantages over conventional plasmid DNA, such as simplicity in purification, absence of endotoxin contamination, and more importantly, flexibility in chemical modifications to render them specific properties. We have used PCR amplified fragments as a model to test the feasibility of using synthetic genes for gene therapy. The CAT reporter gene driven by the CMV promoter (CMV-CAT), ie a nuclear expression system, or by the bacteriophage T7 promoter (T7-CAT), ie a cytoplasmic expression system, was used to evaluate this concept. The expression efficiency of both plasmids (pUCCNV-CAT and pT7-CAT) and their corresponding PCR fragments (fCMV-CAT and fT7-CAT) were compared on a molar basis. Limited expression of CAT was found with fCMV-CAT. However, fT7-CAT consistently gave a CAT activity comparable to that of pT7-CAT. When T7-CAT was codelivered with pCMV/T7-T7pol (a self-amplifying T7 RNA-polymerase autogene), high CAT activity could be detected up to 9 days. This expression was much longer than the duration of expression with a nuclear expression system. These encouraging results imply that gene therapy with synthetic genes could be both feasible and efficient.
Descriptors--Author Keywords: gene therapy ; synthetic genes ; polymerase chain reaction ; liposome
Identifiers--KeyWord Plus(R): BACTERIOPHAGE -T7 RNA-POLYMERASE; EXPRESSION SYSTEM; PLASMID DNA; CATIONIC LIPOSOMES; MAMMALIAN-CELLS; ANIMAL-MODELS; SEQUENCE
Research Fronts: 95-0981 001 (HUMAN SOMATIC GENE-THERAPY; AUTOLOGOUS BONE-MARROW TRANSPLANTATION; RETROVIRALLY TRANSDUCED CELLS)
95-5061 001 (STRUCTURAL GENE; GLTC-DEPENDENT REGULATION OF BACILLUS-SUBTILIS GLUTAMATE SYNTHASE EXPRESSION; ARABIDOPSIS TYPE-1 PROTEIN PHOSPHATASE)
95-5460 001 (VACCINIA VIRUS EXPRESSION SYSTEM; HEMAGGLUTININ ENVELOPE PROTEIN ; RECOMBINANT BACULOVIRUS SYNTHESIZING BACTERIOPHAGE -T7 RNA-POLYMERASE)
Cited References:
ANDERSON WF, 1992, V256, P808, SCIENCE
CHEN XZ, 1994, V22, P2114, NUCLEIC ACIDS RES
DUNN JJ, 1988, V68, P259, GENE
ELROYSTEIN O, 1989, V86, P6126, P NATL ACAD SCI USA
ENGELHARDT R, 1991, V51, P2524, CANCER RES
FARHOOD H, 1995, V1235, P289, BIOCHIM BIOPHYS ACTA
FOLGER KR, 1982, V2, P1372, MOL CELL BIOL
GAO X, 1991, V179, P280, BIOCHEM BIOPH RES CO
GAO X, 1994, V200, P1201, BIOCHEM BIOPH RES CO
GAO X, 1995, V2, P710, GENE THER
GAO X, 1993, V21, P2867, NUCLEIC ACIDS RES
LEE RJ, 1996, V271, P8481, J BIOL CHEM
LUNDBERG KS, 1991, V108, P1, GENE
MANTHORPE M, 1993, V4, P419, HUM GENE THER

RAETZ CRH, 1990, V59, P129, ANNU REV BIOCHEM
REDL H, 1993, V187, P330, IMMUNOBIOLOGY
SAMBROOK J, 1989, MOL CLONING LAB MANU
SANKARAN L, 1992, V200, P180, ANAL BIOCHEM
WICKS IP, 1995, V6, P317, HUM GENE THER
ZABNER J, 1995, V270, P18997, J BIOL CHEM

...Abstract: offer many potential advantages over conventional plasmid DNA, such as simplicity in purification, absence of endotoxin contamination, and more importantly, flexibility in chemical modifications to render them specific properties. We have...

...driven by the CMV promoter (CMV-CAT), ie a nuclear expression system, or by the bacteriophage T7 promoter (T7-CAT), ie a cytoplasmic expression system, was used to evaluate this concept...

...pCMV/T7-T7pol (a self-amplifying T7 RNA-polymerase autogene), high CAT activity could be detected up to 9 days. This expression was much longer than the duration of expression with...
...Identifiers-- BACTERIOPHAGE -T7 RNA-POLYMERASE; EXPRESSION SYSTEM; PLASMID DNA; CATIONIC LIPOSOMES; MAMMALIAN-CELLS; ANIMAL-MODELS; SEQUENCE
...Research Fronts: 001 (STRUCTURAL GENE; GLTC-DEPENDENT REGULATION OF BACILLUS-SUBTILIS GLUTAMATE SYNTHASE EXPRESSION; ARABIDOPSIS TYPE-1 PROTEIN PHOSPHATASE)
95-5460 001 (VACCINIA VIRUS EXPRESSION SYSTEM; HEMAGGLUTININ ENVELOPE PROTEIN ; RECOMBINANT BACULOVIRUS SYNTHESIZING BACTERIOPHAGE -T7 RNA-POLYMERASE)

7/9,K/2 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04510165 Genuine Article#: TJ163 Number of References: 14
Title: EXPRESSION OF CARCINOSCORPIAS-ROTUNDICAUDA FACTOR CDNA
Author(s): ROOPASHREE SD; CHAI C; HO B; DING JL
Corporate Source: NATL UNIV SINGAPORE,DEPT ZOOL,10 KENT RIDGE CRESCENT/SINGAPORE 0511//SINGAPORE/; NATL UNIV SINGAPORE,DEPT ZOOL/SINGAPORE 0511//SINGAPORE/; NATL UNIV SINGAPORE,DEPT MICROBIOL/SINGAPORE 0511//SINGAPORE/; NATL UNIV SINGAPORE,CTR BIOSCI/SINGAPORE 0511//SINGAPORE/
Journal: BIOCHEMISTRY AND MOLECULAR BIOLOGY INTERNATIONAL, 1995, V35, N4 (APR), P841-849
ISSN: 1039-9712
Language: ENGLISH Document Type: ARTICLE
Geographic Location: SINGAPORE
Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: The cDNA encoding Factor C (FC) from the Singaporean horseshoe crab Carcinoscorpius rotundicauda has been studied for in vitro coupled transcription-translation (TnT) under the T7 promoter. Two species of full length cDNA, CrFC26 and CrFC21 which differ in length and nucleotide sequence at their 5' untranslated regions (UTR) were used in this study. Wild type CrFC26 with a long 5' UTR containing multiple ''false'' ATGs failed to generate a translated product. With a more accessible ATG codon in CrFC21, the recombinant construct gave a high

yield of FC when transcribed and translated in vitro. CrFC26 deletion mutants which lack the entire 5' UTR and portions of the putative leader peptide were translatable, albeit at lower efficiency as compared to CrFC21. In vitro and in vivo expression of truncated portions of the CrFC21-T7 gene 10 fusions have been compared. In vitro reactions yielded single gene products from each of the expression constructs whereas E. coli produced three major immunoreactive bands of FC.
Identifiers--KeyWords Plus: SERINE-PROTEASE ZYMOGEN; FACTOR-C; RNA-POLYMERASE; BACTERIOPHAGE -T7; HEMOCYTES; PROTEINS
Research Fronts: 93-3088 001 (RAT MUSCLE; PROTEIN PHOSPHATASE-1; MAJOR GLUTATHIONE TRANSFERASE)
93-6725 001 (CHROMOGENIC LIMULUS AMEBOCYTE LYSATE ASSAY; DETECTION OF ENDOTOXIN ; SALMONELLA LIPOPOLYSACCHARIDE)
93-8060 001 (MEMBRANE DOMAIN OF A BACTERIOPHAGE ASSEMBLY PROTEIN ; ESCHERICHIA-COLI K-12; SYNTHETIC GENE; AFRICAN SWINE FEVER VIRUS; PHI-29 DNA-POLYMERASE ACTIVE-SITE)
Cited References:
BURNETTE WN, 1981, V112, P195, ANAL BIOCHEM
CHAMBERLIN M, 1970, V228, P227, NATURE
DING JL, 1993, V1202, P149, BIOCHIM BIOPHYS ACTA
DOHERTY AJ, 1993, V136, P337, GENE
HO B, 1993, V24, P81, MICROBIOS LETT
KOZAK M, 1983, V47, P1, MICROBIOL REV
LAEMMLI UK, 1970, V227, P680, NATURE
MIURA Y, 1992, V112, P476, J BIOCHEM-TOKYO
MUTA T, 1991, V266, P6554, J BIOL CHEM
NAKAMURA T, 1986, V154, P511, EUR J BIOCHEM
NAVAS MAA, 1990, V21, P805, BIOCHEM INT
SEKIGUCHI K, 1979, P37, BIOMEDICAL APPLICATI
STUDIER FW, 1986, V189, P113, J MOL BIOL
STUDIER WF, 1990, V185, P60, METHOD ENZYMOL

...Identifiers--SERINE-PROTEASE ZYMOGEN; FACTOR-C; RNA-POLYMERASE; BACTERIOPHAGE -T7; HEMOCYTES; PROTEINS
Research Fronts: 93-3088 001 (RAT MUSCLE; PROTEIN PHOSPHATASE-1; MAJOR GLUTATHIONE TRANSFERASE)
93-6725 001 (CHROMOGENIC LIMULUS AMEBOCYTE LYSATE ASSAY; DETECTION OF ENDOTOXIN ; SALMONELLA LIPOPOLYSACCHARIDE)
93-8060 001 (MEMBRANE DOMAIN OF A BACTERIOPHAGE ASSEMBLY PROTEIN ; ESCHERICHIA-COLI K-12; SYNTHETIC GENE; AFRICAN SWINE FEVER VIRUS; PHI-29 DNA-POLYMERASE ACTIVE...

7/9,K/3 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

11658508 EMBASE No: 2002231444
Isolation of endotoxin -specific antibodies by selection of an single chain phage antibody library
Chen M.; Yu L.-L.; Zhang X.; Fu W.-L.
W.-L. Fu, Department of Laboratory Diagnosis, South-western Hospital, Third Military Medical University, Chongqing 400038 China
AUTHOR EMAIL: weilingfu@yahoo.com
Chinese Journal of Cancer Research (CHIN. J. CANCER RES.) (China)
2002, 14/2 (118-121)

CODEN: CJCRF ISSN: 1000-9604
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 18

Objective: To isolate murine anti endotoxin single chain phage antibody from a constructed library. Methods: Total RNA was firstly extracted from murine splenic cells and mRNA was reverse-transcribed into cDNA. Then the designed primers were used to amplify the variable region genes of the heavy and light chain (VH, VL) with polymerase chain reaction. The linker was used to assemble the VH and VL into ScFv, and the NotI and SfiI restriction enzymes were used to digest the ScFv in order to ligate into the pCANTAB5E phagemid vector that was already digested with the same restriction enzymes. The ligated vector was then introduced into competent E.coli TG1 cells to construct a single-chain phage antibody library. After rescued with M13KO7 helper phage, recombinant phages displaying ScFv fragments were harvested from the supernatant and selected with endotoxin . The enriched positive clones were reinfected into TG1 cells. Finally, 190 clones were randomly selected to detect the anti endotoxin antibody with indirect ELISA. Results: The titer of anti endotoxin in murine sera was 1:12,800. The concentration of total RNA was 12.38 mug/ml. 1.9x10SUP7 clones were obtained after transformed into TG1. 3x10SUP4 colonies were gotten after one round panning. Two positive colonies were confirmed with indirect ELISA among 190 randomly selected colonies. Conclusion: A 1.9x10SUP7 murine anti endotoxin single chain phage antibody library was successfully constructed. Two anti endotoxin antibodies were obtained from the library.

DRUG DESCRIPTORS:
* endotoxin ; *single chain fragment variable antibody
complementary DNA; restriction endonuclease; protein Notch; unclassified drug
MEDICAL DESCRIPTORS:
*antibody detection ; *antibody engineering
DNA library; gene amplification; molecular cloning; enzyme linked immunosorbent assay; spleen cell; polymerase chain reaction; Escherichia coli; concentration response; bacteriophage ; phagemid; antibody titer; nonhuman; female; mouse; controlled study; animal tissue; article
DRUG TERMS (UNCONTROLLED): protein SfiI; protein Not1
CAS REGISTRY NO.: 334577-34-3, 334577-38-7 (single chain fragment variable antibody)
SECTION HEADINGS:
016 Cancer
026 Immunology, Serology and Transplantation
027 Biophysics, Bioengineering and Medical Instrumentation

Isolation of endotoxin -specific antibodies by selection of an single chain phage antibody library

Objective: To isolate murine anti endotoxin single chain phage antibody from a constructed library. Methods: Total RNA was firstly extracted from ...

...helper phage, recombinant phages displaying ScFv fragments were harvested from the supernatant and selected with endotoxin . The enriched positive clones were reinfected into TG1 cells. Finally, 190 clones were randomly selected to detect the anti endotoxin antibody with indirect

ELISA. Results: The titer of anti endotoxin in murine sera was 1:12,800. The concentration of total RNA was 12.38...

...confirmed with indirect ELISA among 190 randomly selected colonies. Conclusion: A 1.9x10SUP7 murine anti endotoxin single chain phage antibody library was successfully constructed. Two anti endotoxin antibodies were obtained from the library.
DRUG DESCRIPTORS:
* endotoxin ; *single chain fragment variable antibody
complementary DNA; restriction endonuclease; protein Notch; unclassified drug
MEDICAL DESCRIPTORS:
*antibody detection ; *antibody engineering
...molecular cloning; enzyme linked immunosorbent assay; spleen cell; polymerase chain reaction; Escherichia coli; concentration response; bacteriophage ; phagemid; antibody titer; nonhuman; female; mouse; controlled study; animal tissue; article
DRUG TERMS (UNCONTROLLED): protein Sfi1; protein Not1

7/9,K/4 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0415596 DBR Accession No.: 2007-01534 PATENT
Novel nucleic acid encoding polypeptide having hydrolase activity, useful in food supplements, identifying hydrolase modulators, hydrolyzing triacylglycerol, preventing lipopolysaccharide-mediated toxicity, and preparation of propionic acid - transgenic plant, artificial chromosome, transgenic plant for recombinant lipase production for use in paper, pharmaceutical and food industry
AUTHOR: KEROVUO J S; MCCANN R; WEINER D; SOLBAK A I
PATENT ASSIGNEE: DIVERSA CORP 2006
PATENT NUMBER: WO 200696834 PATENT DATE: 20060914 WPI ACCESSION NO.: 2007-008852 (200701)
PRIORITY APPLIC. NO.: US 660122 APPLIC. DATE: 20050308
NATIONAL APPLIC. NO.: WO 2006US8555 APPLIC. DATE: 20060308
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A nucleic acid (NA) encoding a polypeptide having hydrolase activity, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) nucleic acid probe for identifying nucleic acid encoding polypeptide with hydrolase activity; (2) amplification primer pair amplifying SEQ ID No. 1-991 (odd SEQ ID numbers); (3) amplifying (M45) nucleic acid encoding polypeptide with hydrolase activity; (4) expression cassette, vector and cloning vehicle, having nucleic acid; (5) transformed cell having nucleic acid, or expression cassette having nucleic acid; (6) transgenic non-human animal/seed/plant, having the nucleic acid sequence; (7) an antisense oligonucleotide, having nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to (SEQ ID No. 1-991); (8) inhibiting (M46) translation of hydrolase message in cell; (9) a double-stranded inhibitory RNA molecule, having subsequence of nucleic acid; (10) inhibiting (M47) the expression of a hydrolase in a cell; (11) an isolated or recombinant polypeptide (P1); (12) isolated/recombinant polypeptide having (P1) and lacking a signal sequence or having heterologous signal sequence; (13) protein preparation having (P1), where the preparation is

liquid/solid/gel; (14) heterodimer having (P1) and second domain; (15) immobilized polypeptide having (P1); (16) array having an immobilized polypeptide or immobilized nucleic acid; (17) an isolated or recombinant antibody that specifically binds to (P1); (18) hybridoma having an antibody that binds to (P1); (19) food supplement for an animal and edible enzyme delivery matrix having (P1); (20) isolating or identifying (M1) a polypeptide with a hydrolase activity; (21) producing (M2) an anti-hydrolase antibody; (22) producing (M3) a recombinant polypeptide; (23) identifying a polypeptide (M4) having a hydrolase activity or hydrolase substrate; (24) determining (M5) whether a test compound specifically binds to a polypeptide; (25) identifying (M6) a modulator of a hydrolase activity; (26) a computer system having a processor and a data storage device, where the data storage device has stored on it (P1); (27) identifying (M7) a feature in a sequence; (28) comparing (M8) two sequences; (29) isolating a nucleic acid encoding a polypeptide with a hydrolase activity from an environmental sample; (30) generating (M9) a variant of a nucleic acid encoding a hydrolase; (31) modifying (M10) codons in a nucleic acid encoding a hydrolase to increase its expression in a host cell; (32) producing (M11) library of nucleic acids encoding several modified hydrolase active sites/substrate binding sites; (33) making (M12) small molecule; (34) modifying (M13) small molecule; (35) determining (M14) functional fragment of hydrolase enzyme; (36) whole cell engineering (M15) of new/modified phenotypes using real-time metabolic flux analysis; (37) hydrolyzing (M16) triacylglycerol (TAG), diacylglycerol (DAG) or monoacylglycerol (MAG); (38) removing/decreasing (M17) amount of TAG, DAG or MAG from composition; (39) increasing (M18) thermotolerance/thermostability of hydrolase polypeptide; (40) overexpressing (M19) recombinant hydrolase polypeptide in cell; (41) detergent composition having (P1) or polypeptide encoded by the nucleic acid; (42) washing (M20) object; (43) hydrolyzing (M21) oil in feed or food prior to consumption by animal; (44) a feed having (P1) or polypeptide encoded by the nucleic acid; (45) a composition having an oil and the recombinant polypeptide; (46) pharmaceutical containing recombinant polypeptide; (47) making (M22) a transgenic plant; (48) expressing (M23) heterologous nucleic acid sequence in plant cell; (49) signal sequence having a peptide having a subsequence of (P1); (50) chimeric protein having a first domain having the signal sequence and second domain; (51) biocatalytic (M24) synthesis of a structured lipid; (52) preparation (M25) of an optical isomer of a propionic acid from a racemic ester of the propionic acid; (53) stereoselectively (M26) hydrolyzing racemic mixtures of esters of 2-substituted acids; (54) oil or fat modification (M27); (55) hydrolysis (M28) of polyunsaturated fatty acid (PUFA) esters; (56) selective (M29) hydrolysis of PUFA esters over saturated fatty acid esters; (57) preparing (M30) a food or a feed additive having PUFA; (58) treatment (M31) method of latex; (59) refining (M32) a lubricant; (60) treating (M33) a fabric; (61) decreasing (M34) the amount of a food or oil stain; (62) dietary composition having hydrolase; (63) reducing (M35) fat content in milk or vegetable-based dietary compositions; (64) dietary composition; (65) catalyzing (M36) an interesterification reaction to produce new triglycerides; (66) transesterification (M37) method for preparing a margarine oil having a low trans-acid and a low intermediate chain fatty acid content; (67) ameliorating/preventing (M38) lipopolysaccharide- mediated toxicity; (68) detoxifying (M39) an endotoxin ; (69) deacylating (M40) 2' or 3' fatty acid chain from a lipid A; (70) hydrolyzing (M41) composition having a cellulose or

lipophilic compound; (71) making (M42) paper; (72) composition which encoded by nucleic acid; (73) generating (M43) sterol/glycerol/free fatty acid by hydrolyzing composition having cellulose/lipophilic compound; (74) decreasing (M44) amount of lipophilic extract in compound having cellulose; and (75) fabric/yarn/fiber having hydrolase. BIOTECHNOLOGY - Preparation (claimed): The nucleic acid is isolated or recovered from an environmental sample, by providing the amplification primer sequence pair, isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair, and combining the nucleic acid with the primer pair and amplifying nucleic acid from the environmental sample or providing a polynucleotide probe comprising the nucleic acid sequence, isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe, combining the isolated nucleic acid or the treated environmental sample with the polynucleotide probe and isolating a nucleic acid that specifically hybridizes with the polynucleotide probe. The environmental sample comprises water sample, liquid sample, soil sample, air sample or biological sample. The biological sample is derived from bacterial cell, protozoan cell, insect cell, yeast cell, plant cell, fungal cell or mammalian cell. Preferred Nucleic Acid: NA is an isolated or recombinant nucleic acid (a) having at least 51-99% sequence identity to SEQ ID No. 1-991 (odd SEQ ID numbers) over region of 50, 75, 100, 150-1150 (in multiples of 50) or more residues, polypeptide/peptide generating antibody that binds to polypeptide having SEQ ID No. 2-992, and sequence identities are determined by analysis with sequence comparison algorithm or by visual inspection, nucleic acid sequence that hybridizes under stringent conditions to SEQ ID No. 1-991 (odd SEQ ID numbers), and nucleic acid is 20-1000 (in multiples of 10) or more residues in length or full length of gene or transcript, or nucleic acid sequence complementary to it, or (b) sequence that hybridizes to a nucleic acid having SEQ ID NO. 1-991 (odd SEQ ID numbers), where the nucleic acid encodes a polypeptide having a hydrolase activity. The sequence identity is over a region of at least 51-99% or more or is 100% or over a region of at least 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550 or more residues, or the full length of a gene or transcript, preferably 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900,1000 or more residues in length. The nucleic acid sequence comprises a sequence having SEQ ID Nos. 1-991 (odd SEQ ID numbers) or encodes a polypeptide having SEQ ID No. 2-992 (even SEQ ID numbers). The sequence comparison algorithm is a BLAST version 2.2.2 algorithm, where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default. The hydrolase activity includes lipase activity, protease activity, esterase activity or phospholipase activity. The lipase activity involves hydrolyzing a triacylglycerol to a diacylglycerol and a free fatty acid or hydrolyzing a triacylglycerol to a monoacylglycerol and free fatty acids or hydrolyzing a diacylglycerol to a monoacylglycerol and free fatty acids, or hydrolyzing monoacylglycerol to free fatty acid and glycerol, or hydrolyzing triacylglycerol, diacylglycerol or monoacylglycerol or synthesizing a tryacylglycerol from diacylglycerol or monoacylglycerol and free fatty acids. The lipase activity involves synthesizing 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1,3- distearoyl-2-oleoylglycero

l (SOS), l-palmitoyl-2-oleoyl-3-stearoyl glycerol (POS) or 1-oleoyl-2,3-dimyristoylglycerol (OMM), long chain polyunsaturated fatty acids, arachidonic acid, docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA). The lipase activity is triacylglycerol, diacylglycerol or monoacylglycerol position specific. The lipase activity is Sn2-specific, Sn1- or Sn3-specific or fatty acid specific. The lipase activity comprises modifying oils by hydrolysis, alcoholysis, esterification, transesterification or interesterification. The lipase activity is regio-specific or chemoselective. The lipase activity comprises synthesis of enantiomerically pure chiral products. The lipase activity comprises synthesis of umbelliferyl fatty acid (FA) esters. The lipase activity is thermostable. The polypeptide retains a lipase activity under conditions comprising a temperature range of 37-95degreesC, 55-85degreesC, 70-95degreesC, or 90-95degreesC. The hydrolase activity is thermotolerant. The polypeptide retains a hydrolase activity after exposure to a temperature in the range from greater than 37-95degreesC, 55-85degreesC, or 90-95degreesC. The stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of 65degreesC for 15 minutes. Preferred Polypeptide: P1 has at least 50-99% or more identity to SEQ ID No. 2-992 (even SEQ ID numbers), over a region of at least 20-700 (in multiples of 10) or more residues, where optinally the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or encoded by the nucleic acid; Preferred Probe: The probe comprises 10 consecutive bases of SEQ ID No. 1-991 (odd SEQ ID numbers), where the probe identifies the nucleic acid by binding or hybridization and the sequence identities are determined by analysis with sequence comparison algorithm or by visual inspection Preferred Cloning Vehicle: The cloning vehicle comprises viral vector, plasmid, phage, phagemid, cosmid, fosmid, bacteriophage or artificial chromosome. The viral vector comprises adenovirus vector, retroviral vector or adeno-associated viral vector or comprises bacterial artificial chromosome (BAC), plasmid, bacteriophage P1-derived vector (PAC), yeast artificial chromosome (YAC), or mammalian artificial chromosome (MAC). Preferred Host Cell: The cell is bacterial cell, mammalian cell, fungal cell, yeast cell, insect cell or plant cell, preferably potato, rice, corn, wheat, tobacco or barley cell. Preferred Transgenic Animal: The animal is a mouse. Preferred Transgenic Plant: The plant is corn plant, sorghum plant, potato plant, tomato plant, wheat plant, oilseed plant, rapeseed plant, soybean plant, rice plant, barley plant, grass, or tobacco plant. Preferred Transgenic Seed: The seed is rice, corn seed, wheat kernel, oilseed, rapeseed, soybean seed, palm kernel, sunflower seed, sesame seed, rice, barley, peanut or tobacco plant seed. Preferred Heterodimer: The second domain is a polypeptide and the heterodimer is a fusion protein or epitope or tag. Preferred Matrix: The delivery matrix comprises a pellet. Preferred Method: Isolating or identifying (M1) a polypeptide with a hydrolase activity, involves providing isolated or recombinant antibody, sample comprising polypeptides, and contacting the sample with the antibody under conditions, where the antibody can specifically bind to the polypeptide, thus isolating or identifying a polypeptide having a hydrolase activity. Producing (M2) anti-hydrolase antibody, involves administering to a non-human animal the nucleic acid or (P1) to generate a humoral immune response, thus making an anti-hydrolase antibody. Producing (M3) a recombinant polypeptide, involves providing the nucleic acid operably linked to a promoter, and expressing the nucleic acid under conditions that allow expression of the polypeptide.

Identifying (M4) a polypeptide having a hydrolase activity or hydrolase substrate, involves providing a recombinant polypeptide, hydrolase substrate, and contacting the polypeptide with the substrate and detecting a decrease in the amount of substrate or increase in the amount of a reaction product, where decrease in the amount of the substrate or increase in the amount of the reaction product detects a polypeptide having a hydrolase activity or where a decrease in amount of substrate or increase in amount of reaction product identifies test substrate as a hydrolase substrate. The substrate is fatty acid, TAG, DAG or MAG. Determining (M5) whether a test compound specifically binds to a polypeptide, involves expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, where the nucleic acid has SEQ ID No. 1-991 (odd SEQ ID numbers), or providing (P1), and providing a test compound, contacting the polypeptide with the test compound and determining whether test compound specifically binds to polypeptide. Identifying (M6) a modulator of a hydrolase activity, involves providing the recombinant polypeptide, test compound, contacting the polypeptide with the test compound and measuring hydrolase activity, where a change in activity measured in presence of test compound compared to activity in absence provides a determination that the test compound modulates hydrolase activity. Identifying (M7) a feature in a sequence, involves reading the sequence using a computer program which identifies one or more features in a sequence, where the sequence comprises (P1) or a nucleic acid sequence, a polypeptide encoded by the nucleic acid and identifying one or more features in the sequence with the computer program. Comparing (M8) a first sequence to a second sequence, involves reading the first sequence and the second sequence through use of a computer program which compares sequences, where the first sequence comprises a (P1) or a nucleic acid sequence, or a polypeptide encoded by the nucleic acid and determining differences between first and second sequence with the computer program. Generating (M9) a variant of a nucleic acid encoding a polypeptide with a hydrolase activity, involves providing a template nucleic acid comprising the nucleic acid sequence and modifying, deleting or adding one or more nucleotides in the template sequence, or their combination. Modifying (M10) codons in a nucleic acid encoding a polypeptide with a hydrolase activity to increase its expression in a host cell, involves providing a nucleic acid encoding a polypeptide with a hydrolase activity comprising the nucleic acid sequence and identifying a non-preferred or a less preferred codon in the nucleic acid and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, where a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thus modifying the nucleic acid to increase its expression in a host cell or identifying a codon in the nucleic acid and replacing it with a different codon encoding the same amino acid as the replaced codon, thus modifying codons in a nucleic acid encoding a hydrolase or identifying at least one preferred codon in the nucleic acid and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, where a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell. Producing (M11) a library of nucleic acids encoding a plurality of modified hydrolase active sites or substrate binding sites, where

the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site, involves providing a first nucleic acid encoding a first active site or first substrate binding site, where the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to the nucleic acid sequence, and the nucleic acid encodes a hydrolase active site or a hydrolase substrate binding site, mutagenic oligonucleotides that encode naturally-occurring amino acid variants at several targeted codons in the first nucleic acid and using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized. The host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell. Making (M12) a small molecule, involves providing several biosynthetic enzymes capable of synthesizing or modifying a small molecule, where one of the enzymes comprises a hydrolase enzyme encoded by the nucleic acid, substrate for enzymes, and reacting the substrate with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions. Modifying (M13) a small molecule, involves providing a hydrolase enzyme, where the enzyme comprises the recombinant polypeptide, or a polypeptide encoded by the nucleic acid sequence, small molecule and reacting the enzyme with the small molecule that facilitate enzymatic reaction catalyzed by the hydrolase enzyme. Determining (M14) a functional fragment of a hydrolase enzyme, involves providing a hydrolase enzyme comprising the recombinant polypeptide, or polypeptide encoded by the nucleic acid and deleting several amino acid residues and testing the remaining subsequence for a hydrolase activity. Method (M15) for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, involves making a modified cell by modifying the genetic composition of a cell, where the genetic composition is modified by addition to the cell of the nucleic acid, culturing the modified cell, measuring metabolic parameter of the cell by monitoring the cell culture in real time and analyzing the data to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thus identifying an engineered phenotype in the cell using real-time metabolic flux analysis. Hydrolyzing (M16) a triacylglycerol, diacyl glycerol or monoacylglycerol, involves providing the polypeptide having a hydrolase activity, or polypeptide encoded by the nucleic acid,composition comprising triacyl glycerol, diacyl glycerol or monoacylglycerol, and contacting the polypeptide with the composition, where the polypeptide hydrolyzes triacylglycerol, diacylglycerol or monoacylglycerol. Removing (M17) or decreasing the amount of triacylglycerol, diacylglycerol or monoacylglycerol from a composition, involves providing the recombinant polypeptide having a hydrolase activity, or a polypeptide encoded by the nucleic acid, providing a composition comprising a triacylglycerol, diacylglycerol or monoacylglycerol, and contacting the polypeptide with the composition, where the polypeptide removes or decreases the amount of the triacylglycerol, diacylglycerol or monoacylglycerol. Increasing (M18) thermotolerance or thermostability of a hydrolase polypeptide, involves glycosylating a hydrolase polypeptide, where the polypeptide comprises at least thirty contiguous amino acids of (P1) or a polypeptide encoded by the nucleic acid. The hydrolase specific activity is thermostable or thermotolerant

at a temperature in range greater than 37-95degreesC. Overexpressing (M19) a recombinant hydrolase polypeptide in a cell, involves expressing a vector comprising the nucleic acid sequence, where overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector. Washing (M20) an object, involves providing a composition comprising the recombinant polypeptide having a hydrolase activity, or a polypeptide encoded by the nucleic acid, providing an object, and contacting the polypeptide and the object under conditions where the composition can wash the object. Hydrolyzing (M21) an oil in a feed or a food prior to consumption by an animal, involves obtaining a feed material comprising an oil, where the oil can be hydrolyzed by the recombinant polypeptide having a hydrolase activity, or a polypeptide encoded by the nucleic acid, and adding the polypeptide to the feed or food material for a sufficient period to cause hydrolysis of the oil and formation of a treated food or feed. The food or feed comprises rice, corn, barley, wheat, legumes or potato. Making (M22) a transgenic plant, involves introducing a heterologous nucleic acid sequence into the cell, where the heterologous nucleic sequence comprises SEQ ID No. 1-991 (odd SEQ ID numbers) and producing a transgenic plant from the transformed cell. Expressing (M23) a heterologous nucleic acid sequence in a plant cell, involves transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, and growing the plant under conditions, where the heterologous nucleic acids sequence is expressed in the plant cell. Biocatalytic (M24) synthesis of a structured lipid, involves providing the recombinant polypeptide having hydrolase and composition comprising triacylglyceride, contacting the polypeptide with the composition under conditions, where the polypeptide hydrolyzes acyl residue at the Sn2 position or Sn1 or Sn3 of triacylglyceride, thus producing a 1,3-diacylglyceride or 1,2-diacylglyceride or 2,3-diacylglyceride and promoting acyl migration under kinetically controlled conditions, providing an R1 ester, R1-specific hydrolase and contacting the 1,3-DAG with the R1 ester and the R1-specific hydrolase, where the R1-specific hydrolase catalyzes esterification of the Sn2 position, thus producing the structured lipid. the lipid includes cocoa butter alternative (CBA), synthetic cocoa butter, natural cocoa butter, 1,3- dipalmitoyl-2-oleoylglycerol (POP), 1,3-distearoyl-2-oleoylglycerol (SOS), 1-palmitoyl- 2-oleoyl-3-stearoylglycerol (POS) or 1-oleoyl-2,3-dimyristoylglycerol (OMM). Preparation (M25) of an optical isomer of a propionic acid from a racemic ester of the propionic acid, involves providing a hydrolase being stereoselective for an optical isomer of the propionic acid, racemic esters contacting the polypeptide with the racemic esters, where the polypeptide can selectively catalyze the hydrolysis of the esters, thus producing the optical isomer of the propionic acid. Stereoselectively (M26) hydrolyzing racemic mixtures of esters of 2-substituted acids, involves providing a hydrolase the stereoselective, composition comprising a racemic mixture of esters of 2- substituted acids and contacting the polypeptide with the composition under conditions, where the polypeptide can selectively hydrolyze the esters. Method (M27) for oil or fat modification, involves providing a hydrolase, oil or fat, and contacting the hydrolase with the oil or fat under conditions, where the hydrolase can modify the oil or fat. The oil comprises a glycerol ester of PUFA, or fish, animal or vegetable oil. Hydrolysis (M28) of polyunsaturated fatty acid (PUFA) esters, involves providing a hydrolase, composition comprising PUFA esters, and contacting the hydrolase with the composition under conditions, where the hydrolase can hydrolyze PUFA

ester. Selective (M29) hydrolysis of PUFA esters over saturated fatty acid esters, involves providing a hydrolase having a lipase activity and selectively hydrolyzes PUFA esters, composition comprising a mixture of polyunsaturated and saturated esters, and contacting the polypeptide with the composition under conditions, where the polypeptide can selectively catalyze the hydrolysis of PUFA esters. Preparing (M30) a food or a feed additive comprising PUFA, involves providing a hydrolase that selectively hydrolyzes PUFA esters, composition comprising a PUFA ester, and contacting the polypeptide with the composition under conditions, where the polypeptide can selectively catalyze the hydrolysis of PUFA esters, thus producing the PUFA-containing food or feed additive. Treatment (M31) of latex, involves providing a hydrolase having selectivity for a saturated ester over an unsaturated ester, thus converting the saturated ester to its corresponding acid and alcohol, a latex composition comprising saturated and unsaturated esters contacting the hydrolase with the composition under conditions where the polypeptide can selectively hydrolyze saturated esters, thus treating the latex. Refining (M32) a lubricant, involves providing a composition comprising hydrolase and lubricant, treating the lubricant with the hydrolase under conditions, where the hydrolase can selective hydrolyze oils in the lubricant, thus refining it. Treating (M33) a fabric, involves providing a composition comprising hydrolase that selectively hydrolyze carboxylic esters, and providing a fabric, treating the fabric with the hydrolase under condition, where the hydrolase can selectively hydrolyze carboxylic esters, thus treating the fabric. Removing (M34) or decreasing the amount of a food or oil stain, involves contacting a hydrolase with the food or oil stain under conditions, where the hydrolase can hydrolyze oil or fat in the stain. Reducing (M35) fat content in milk or vegetable-based dietary compositions, involves providing a composition comprising a hydrolase, milk or vegetable oil, and treating the composition with the hydrolase under conditions, where the hydrolase can hydrolyze the oil or fat in the composition, thus reducing its fat content. Catalyzing (M36) an interesterification reaction to produce new triglycerides, involves providing a composition comprising a hydrolase that can catalyze an interesterification reaction, providing a mixture of triglycerides and free fatty acids, treating the composition with the hydrolase under conditions, where the hydrolase can catalyze exchange of free fatty acids with the acyl groups of triglycerides, thus producing new triglycerides enriched in the added fatty acids. Transesterification (M37) method for preparing a margarine oil having a low trans-acid and a low intermediate chain fatty acid content, involves providing a transesterification reaction mixtu

DESCRIPTORS: recombinant hydrolase-like protein , lipase prep., plasmid, phage, phagemid, cosmid, fosmid, adeno virus, retro virus, adeno-associated virus vector, bacterial artificial chromosome, P1-derived vector, yeast artificial chromosome, mammal artificial chromosome-mediated gene transfer, expression in bacterium cell, mammal cell, fungus cell, yeast cell, insect cell, plant cell, transgenic mouse, maize, sorghum, potato, tomato, wheat, oilseed, rapeseed oil, soybean, rice, barley, grass, tobacco transgenic plant, seed, DNA probe, DNA primer, DNA array, protein array, recombinant antibody, hybridoma, computer, phenotyping, polyunsaturated fatty acid ester hydrolysis, oil, fat modification, transesterification, algorithm, appl., foodstuff, feedstuff, margarine oil prep., stereospecific, pharmaceutical, paper ind. enzyme hybridization DNA amplification cell culture culture esterification virus parvo virus arthropod animal

transgenic animal mammal plant cereal grass Zea mays Solanum tuberosum fruit Lycopersicon esculentum Triticum aestivum oilseed legume Glycine max Oryza sativa Hordeum vulgare Nicotiana tabacum EC-3.1.1.3 DNA sequence (26, 03)

SECTION: BIOMANUFACTURING and BIOCATALYSIS-Biocatalyst Isolation and Characterization-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; GENETIC TECHNIQUES and APPLICATIONS-Transgenic Animals and Animal Models-BIOINFORMATICS and ANALYSIS-Software; BIOINFORMATICS and ANALYSIS-Biochips and Bioarrays-AGRICULTURAL BIOTECHNOLOGY-Plant Genetic Engineering; FOOD and FOOD-ADDITIVES-Food and Food-Additives-BIOMANUFACTURING and BIOCATALYSIS-Animal/Plant Cell Culture; BIOMANUFACTURING and BIOCATALYSIS-Biocatalyst Application-PHARMACEUTICALS-Other Pharmaceuticals

...ABSTRACT: recombinant polypeptide having (P1) and lacking a signal sequence or having heterologous signal sequence; (13) protein preparation having (P1), where the preparation is liquid/solid/gel; (14) heterodimer having (P1) and...

... plant cell; (49) signal sequence having a peptide having a subsequence of (P1); (50) chimeric protein having a first domain having the signal sequence and second domain; (51) biocatalytic (M24) synthesis...

... chain fatty acid content; (67) ameliorating/preventing (M38) lipopolysaccharide- mediated toxicity; (68) detoxifying (M39) an endotoxin ; (69) deacylating (M40) 2' or 3' fatty acid chain from a lipid A; (70) hydrolyzing...

... inspection Preferred Cloning Vehicle: The cloning vehicle comprises viral vector, plasmid, phage, phagemid, cosmid, fosmid, bacteriophage or artificial chromosome. The viral vector comprises adenovirus vector, retroviral vector or adeno-associated viral vector or comprises bacterial artificial chromosome (BAC), plasmid, bacteriophage P1-derived vector (PAC), yeast artificial chromosome (YAC), or mammalian artificial chromosome (MAC). Preferred Host...

... seed. Preferred Heterodimer: The second domain is a polypeptide and the heterodimer is a fusion protein or epitope or tag. Preferred Matrix: The delivery matrix comprises a pellet. Preferred Method: Isolating...

... involves providing a recombinant polypeptide, hydrolase substrate, and contacting the polypeptide with the substrate and detecting a decrease in the amount of substrate or increase in the amount of a reaction...

...in the amount of the substrate or increase in the amount of the reaction product detects a polypeptide having a hydrolase activity or where a decrease in amount of substrate or...

DESCRIPTORS: recombinant hydrolase-like protein , lipase prep., plasmid, phage, phagemid, cosmid, fosmid, adeno virus, retro virus, adeno-associated virus vector...

...oil, soybean, rice, barley, grass, tobacco transgenic plant, seed, DNA probe, DNA primer, DNA array, protein array, recombinant antibody, hybridoma, computer, phenotyping, polyunsaturated fatty acid ester hydrolysis, oil, fat modification, transesterification...

7/9,K/5 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0385455 DBR Accession No.: 2005-31161 PATENT
NewMut isolated or recombinant polypeptide having phospholipase activity, useful for degumming oil, caustic refining of a phospholipid-containing composition, purifying a phytosterol or a triterpene, or refining a crude oil - production of a recombinant phospholipase and use of the encoding gene for preparation of a transgenic animal or a transgenic plant and for a bioremediation application
AUTHOR: GRAMATIKOVA S; HAZLEWOOD G; LAM D; BARTON N R; STURGIS B G; ROBERTSON D E; LI J; KREPS J A; FIELDING R; BROWN R C; VASAVADA A; TAN X; BADILLO A; VAN HOEK W P; JANSSEN G; ISAAC C; BURK M J
PATENT ASSIGNEE: DIVERSA CORP 2005
PATENT NUMBER: WO 200586900 PATENT DATE: 20050922 WPI ACCESSION NO.: 2005-746919 (200576)
PRIORITY APPLIC. NO.: US 796907 APPLIC. DATE: 20040308
NATIONAL APPLIC. NO.: WO 2005US7908 APPLIC. DATE: 20050308
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated or recombinant polypeptide having phospholipase activity having comprising any one of the even-numbered amino acid sequences of SEQ ID Nos. 2-174, fully defined in the specification, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) an isolated or recombinant nucleic acid comprising a nucleic acid sequence having at least 50% sequence identity to any one of the odd-numbered nucleotide sequence of SEQ ID Nos. 1-174, fully defined in the specification, and encoding the polypeptide cited above; (2) a nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a phospholipase activity; (3) an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a phospholipase activity; (4) an isolated or recombinant phospholipase encoded by a phospholipase-encoding nucleic acid; (5) a method of amplifying a nucleic acid encoding a polypeptide having a phospholipase activity; (6) a method for making a phospholipase; (7) an expression cassette comprising the nucleic acid; (8) a vector comprising the nucleic acid; (9) a cloning vehicle comprising the nucleic acid; (10) a transformed cell comprising the nucleic acid or expression cassette; (11) a transgenic non-human animal comprising the nucleic acid; (12) a transgenic plant or seed comprising the nucleic acid; (13) an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to the nucleic acid sequence cited above or its subsequence; (14) a method of inhibiting the translation of a phospholipase message in a cell; (15) a double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of the nucleotide sequences; (16) a method of inhibiting the expression of a phospholipase in a cell; (17) a protein preparation comprising the polypeptide, where the protein preparation comprises a liquid, a solid or a gel; (18) a heterodimer comprising the polypeptide, and a second domain; (19) a homodimer comprising the polypeptide, or its subsequence; (20) an array comprising an immobilized polypeptide, or nucleic acid;(21) an isolated or recombinant antibody that specifically binds to the polypeptide; (22) a hybridoma comprising an antibody that specifically binds to the polypeptide; (23) a method of isolating or identifying a polypeptide with a phospholipase activity; (24) a method of making an

anti-phospholipase antibody; (25) a method of producing a recombinant polypeptide; (26) a method for identifying a polypeptide having a phospholipase activity; (27) a method for identifying a phospholipase substrate; (28) a method of determining whether a test compound specifically binds to a polypeptide; (29) a method for identifying a modulator of a phospholipase activity; (30) a computer system comprising a processor and a data storage device stored with the polypeptide or nucleic acid sequences cited above;(31) a computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence; (32) a method for identifying a feature in a sequence; (33) a method for comparing a first sequence to a second sequence; (34) a method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample; (35) a method of generating a variant of a nucleic acid encoding a polypeptide with a phospholipase activity; (36) a method for modifying codons in a nucleic acid encoding a polypeptide with a phospholipase activity to increase its expression in a host cell; (37) a method for producing a library of nucleic acids encoding modified phospholipase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site; (38) a method for making or modifying a small molecule; (39) a method for determining a functional fragment of a phospholipase enzyme; (40) a method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis; (41) a chimeric polypeptide comprising at least a first domain comprising signal peptide (SP), and at least a second domain comprising a heterologous polypeptide or peptide, where the heterologous polypeptide or peptide is not naturally associated with the SP; (42) an isolated or recombinant nucleic acid encoding a chimeric polypeptide; (43) a method of increasing thermotolerance or thermostability of a phospholipase polypeptide; (44) a method for overexpressing a recombinant phospholipase in a cell; (45) a method of making a transgenic plant; (46) a method of expressing a heterologous nucleic acid sequence in a plant cell; (47) a method for hydrolyzing, breaking up or disrupting a phospholipid-comprising composition; (48) a method for liquefying or removing a phospholipid-comprising composition; (49) a detergent composition comprising the polypeptide; (50) a method for washing an object; (51) a method for degumming an oil; (52) a method for converting a non-hydratable phospholipid to a hydratable form; (53) a method for caustic refining of a phospholipid-containing composition; (54) a method for purification of a phytosterol or a triterpene; (55) a method for refining a crude oil; (56) a composition having the equivalent of a phospholipase C activity comprising providing a composition comprising a polypeptide having a phospholipase activity, e.g. a phospholipase D activity, and a phosphatase enzyme; (57) a method for ameliorating or preventing lipopolysaccharide (LPS)-mediated toxicity; (58) a method for detoxifying an endotoxin ; (59) a method for deacylating a 2' or a 3 ' fatty acid chain from a lipid A; (60) a process for reducing gum mass and increasing neutral oil (triglyceride) gain through reduced oil entrapment; (61) a method for making a variant phospholipase coding sequence having increased expression in a host cell; (62) a method for making a variant phospholipase coding sequence encoding a phospholipase having increased or decreased resistance to a protease; (63) an isolated, synthetic or recombinant phospholipase encoded by the sequence made as above; (64) a method for making and expressing a

protein having a biological activity whose activity is temporarily inactivated by glycosylation;(65) a method for expressing phospholipase C; (66) a cell system for expressing phospholipase C comprising a Mut+ phenotype Pichia strain comprising a heterologous phospholipase C-encoding nucleic acid operably linked to a promoter operable in the Pichia strain; and (67) a zeocin-resistant yeast cell system for expressing a heterologous protein . BIOTECHNOLOGY - Preferred Molecule: The isolated or recombinant nucleic acid comprises at least 50-100% sequence identity to those nucleotide sequences cited above. The sequence identity is over a region of at least about 10, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950,1000, 1050, 1100, 1150 or more residues, or the full length of a gene or a transcript. The sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default. The phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage, or of an ester linkage in a phospholipid in a vegetable oil. The vegetable oil phospholipid comprises an oilseed phospholipid. The phospholipase activity comprises a phospholipase C (PLC), phospholipase A (PLA), phospholipase B (PLB), or phospholipase D (PLD) activity. The phospholipase D activity comprises a phospholipase D1 or a phospholipase D2 activity. The phospholipase activity comprises hydrolysis of a glycoprotein . The glycoprotein comprises a potato tuber. The phospholipase activity comprises a patatin enzymatic activity, or lipid acyl hydrolase (LAH) activity. The phospholipase activity is thermostable. The polypeptide retains a phospholipase activity under conditions comprising a temperature range of 37-95, 55-85, 70-75, 70-95 or 90-85degreesC. The phospholipase activity is thermotolerant. The nucleic acid hybridizes to the above sequences at stringent conditions including a wash step comprising a wash in 0.2 X SSC at a temperature of 65degreesC for 15 minutes. The phospholipase-encoding nucleic acid is generated by amplification through polymerase chain reaction (PCR), or amplification of a gene library, specifically an environmental library. Preferred Probe: The nucleic acid probe comprises at least 10 consecutive bases of any one of the odd-numbered nucleotide sequences for SEQ ID Nos. 1-173, where the probe identifies the nucleic acid by binding or hybridization. The probe comprises an oligonucleotide comprising at least 10-50, 20-60, 30-70, 40-80, 60-100 or 50-150 consecutive bases. The sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection. The double-stranded inhibitory RNA (RNAi) molecule is 15-25 or more duplex nucleotides in length. Preferred Primer Pair: The amplification primer sequence pair is capable of amplifying the nucleic acids cited above or their subsequence. A member of the amplification primer sequence pair comprises an oligonucleotide comprising at least 10-50 consecutive bases, or about 12-25 consecutive bases of the sequence. The primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12-30 residues of the nucleotide sequences cited above, and a second member having a sequence as set forth by about the first (the 5') 12-30 or more residues of the complementary strand of the first member. Preferred Vehicle: The cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector. The cloning vehicle comprises a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast

artificial chromosome (YAC), or a mammalian artificial chromosome (MAC). Preferred Cell: The transformed cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell. Preferred Animal: The transgenic non-human animal is a mouse. Preferred Plant: The transgenic plant is a corn , a sorghum , a potato , a tomato , a wheat , an oilseed , a rapeseed , a soybean , a rice , a barley , a grass, a cottonseed, a palm, a sesame , a peanut , a sunflower or a tobacco plant. Preferred Polypeptide: The isolated or recombinant polypeptide has the phospholipase activity. The phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage, or catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil. The vegetable oil phospholipid comprises an oilseed phospholipid. The vegetable oil phospholipid is derived from a plant oil, a high phosphorus oil, a soy oil, a canola oil, a palm oil, a cottonseed oil, a corn oil, a palm kernel-derived phospholipid, a rice bran oil, a coconut oil, a peanut oil, a sesame oil, a fish oil, an algae phospholipid, a sunflower oil, an essential oil, a fruit seed oil, a grapeseed phospholipid, an apricot phospholipid, or a borage phospholipid. The isolated or recombinant polypeptide lacks a signal sequence. The polypeptide has a heterologous signal sequence. The phospholipase activity comprises a specific activity at 37degreesC in the range from 10-100, 100-1000, 500-750, 500-1200, or 750-1000 units per milligram. The thermotolerance comprises retention of at least half of the specific activity of the phospholipase at 37degreesC after being heated to an elevated temperature. The polypeptide comprises at least one glycosylation site. The glycosylation is an N-linked glycosylation. The polypeptide is glycosylated after being expressed in P. pastoris or an S. pombe. The polypeptide retains a phospholipase activity under conditions comprising about pH 6.5, pH 6.0, pH 5.5, 5.0, pH 4.5 or 4.0. The immobilized polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube. Isolating or identifying a polypeptide with a phospholipase activity comprises providing the antibody; providing a sample comprising polypeptides; and contacting the sample with the antibody under conditions where the antibody can specifically bind to the polypeptide, thus isolating or identifying a polypeptide having a phospholipase activity. Preferred Method: Amplifying a nucleic acid encoding a polypeptide having a phospholipase activity comprises amplifying a template nucleic acid with the amplification primer sequence pair. Making a phospholipase comprises amplifying a nucleic acid with the amplification primer pair, and expressing the amplified nucleic acid. Inhibiting the translation of a phospholipase message in a cell comprises administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to the nucleotides sequence cited above. Inhibiting the expression of a phospholipase in a cell comprises administering to the cell or expressing in the cell the double-stranded inhibitory RNA (iRNA), Making an anti-phospholipase antibody comprises administering to a non-human animal the nucleic acid/polypeptide or its subsequence in an amount sufficient to generate a humoral immune response, making an anti-phospholipase antibody. Producing a recombinant polypeptide comprises providing the nucleic acid operably linked to a promoter, and expressing the nucleic acid under conditions that allow expression of the polypeptide, thus producing a recombinant polypeptide. The method further comprises transforming a host cell with

the nucleic acid in the first step followed by expressing the nucleic acid. Identifying a polypeptide having a phospholipase activity comprises providing the polypeptide; providing a phospholipase substrate; and contacting the polypeptide with the substrate and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, where a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a phospholipase activity. Identifying a phospholipase substrate comprises providing the polypeptide; providing a test substrate; and contacting the polypeptide with the test substrate and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, where a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a phospholipase substrate. Determining whether a test compound specifically binds to a polypeptide comprises expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide; providing a test compound; contacting the polypeptide with the test compound; and determining whether the test compound specifically binds to the polypeptide. Identifying a modulator of a phospholipase activity comprises providing the polypeptide; providing a test compound; contacting the polypeptide with the test compound; and measuring an activity of the phospholipase, where a change in the phospholipase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the phospholipase activity. The phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product. A decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of phospholipase activity. An increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of phospholipase activity. Identifying a feature in a sequence comprises reading the sequence using a computer program, which identifies one or more features in a sequence, and identifying one or more features in the sequence with the computer program. Comparing a first sequence to a second sequence comprises reading the first sequence and the second sequence through use of a computer program which compares sequences, and determining differences between the first sequence and the second sequence with the computer program. Determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms. The method further comprises an identifier that identifies one or more features in a sequence. The method comprises reading the first sequence using a computer program and identifying one or more features in the sequence. Isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprises providing an amplification primer sequence pair; isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and combining the nucleic acid with the

amplification primer pair; and amplifying nucleic acid from the environmental sample, thus isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample. The environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample. The biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell. Generating a variant of a nucleic acid encoding a polypeptide with a phospholipase activity comprises providing a template nucleic acid; and modifying, deleting or adding one or more nucleotides in the template sequence, or their combination, to generate a variant of the template nucleic acid. The method further comprises expressing the variant nucleic acid to generate a variant phospholipase polypeptide. The modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, Gene Site Saturation Mutagenesis (GSSM), synthetic ligation reassembly (SLR) and their combination. The modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis , artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and their combination. The method is iteratively repeated until a phospholipase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced. The variant phospholipase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature. The variant phospholipase polypeptide has increased glycosylation as compared to the phospholipase encoded by a template nucleic acid. The variant phospholipase polypeptide has a phospholipase activity under a high temperature, where the phospholipase encoded by the template nucleic acid is not active under the high temperature. The method is iteratively repeated until a phospholipase coding sequence having an altered codon usage from that of the template nucleic acid is produced. The method is iteratively repeated until a phospholipase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced. Modifying codons in a nucleic acid encoding a polypeptide with a phospholipase activity to increase its expression in a host cell comprises providing a nucleic acid encoding a polypeptide with a phospholipase activity; identifying a non-preferred or a less preferred codon in the nucleic acid; and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, where a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thus modifying the nucleic acid to increase its expression in a host cell. Modifying codons in a nucleic acid encoding a phospholipase polypeptide comprises providing a nucleic acid encoding a polypeptide with a phospholipase activity; and identifying a codon in the nucleic acid and replacing it with a

different codon encoding the same amino acid as the replaced codon. Modifying codons in a nucleic acid encoding a phospholipase polypeptide to increase or decrease its expression in a host cell comprises providing a nucleic acid encoding a phospholipase polypeptide; and identifying a non-preferred or a less preferred codon in the nucleic acid and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, where a preferred codon is a codon over-represented or under-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell. Producing a library of nucleic acids encoding modified phospholipase active sites or substrate binding sites comprises providing a first nucleic acid encoding a first active site or first substrate binding site, providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at targeted codons in the first nucleic acid; and using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized. The method comprises mutagenizing the first nucleic acid or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis , artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and their combination. Making a small molecule comprises providing biosynthetic enzymes capable of synthesizing or modifying a small molecule; providing a substrate for at least one of the enzymes; and reacting the substrate with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reaction Modifying a small molecule comprises providing a phospholipase enzyme; providing a small molecule; and reacting the enzyme with the small molecule under conditions that facilitate an enzymatic reaction catalyzed by the phospholipase enzyme. The method further comprises testing the library to determine if a particular modified small molecule, which exhibits a desired activity is present within the library. Determining a functional fragment of a phospholipase enzyme comprises providing a phospholipase enzyme; and deleting amino acid residues from the sequence and testing the remaining subsequence for a phospholipase activity. The method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis comprises making a modified cell by modifying the genetic composition of a cell, where the genetic composition is modified by addition to the cell of a nucleic acid; culturing the modified cell to generate a plurality of modified cells; measuring at least one metabolic parameter of the cell by monitoring the cell culture in real time; and analyzing the data to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions. The genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene. The method further comprises selecting a cell comprising a newly engineered phenotype. The method further comprises culturing the selected cell. Increasing thermotolerance or thermostability of a phospholipase polypeptide

comprises glycosylating a phospholipase. Overexpressing a recombinant phospholipase in a cell comprises expressing a vector comprising a nucleic acid sequence, where overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector. Making a transgenic plant comprises introducing a heterologous nucleic acid sequence into the cell, where the heterologous nucleic sequence, thus producing a transformed plant cell; producing a transgenic plant from the transformed cell. The method further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts. The method comprises introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment or by using an Agrobacterium tumefaciens host. Expressing a heterologous nucleic acid sequence in a plant cell comprises transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter; and growing the plant under conditions where the heterologous nucleic acids sequence is expressed in the plant cell. Hydrolyzing, breaking up or disrupting a phospholipid-comprising composition comprises providing a polypeptide having a phospholipase activity; providing a composition comprising a phospholipid; and contacting the polypeptide with the composition under conditions where the phospholipase hydrolyzes, breaks up or disrupts the phospholipid-comprising composition. Liquefying or removing a phospholipid-comprising composition comprises providing a polypeptide having a phospholipase activity; providing a composition comprising a phospholipid; and contacting the polypeptide with the composition under conditions where the phospholipase removes or liquefies the phospholipid-comprising composition. Washing an object comprises providing a composition comprising a polypeptide having a phospholipase activity; providing an object; and contacting the polypeptide and the object under conditions where the composition can wash the object. Degumming an oil comprises providing a composition comprising a polypeptide having a phospholipase activity; providing a composition comprising a phospholipid-containing fat or oil; and contacting the polypeptide and the composition under conditions where the polypeptide can catalyze the hydrolysis of a phospholipid in the composition. The oil-comprising composition comprises a plant, an animal, algae or a fish oil or fat. The plant oil comprises rice bran oil, soybean oil, a rapeseed oil, a corn oil, oil from a palm kernel, canola oil, sunflower oil, sesame oil or a peanut oil. The polypeptide hyd

DESCRIPTORS: recombinant phospholipase prep., isol., immobilization, bacterium artificial chromosome, plasmid, phage, yeast artificial chromosome, mammal artificial chromosome vector-mediated gene transfer, expression in host cell, computer bioinformatic hardware, computer bioinformatic software, maize, sorghum, potato, tomato, wheat, oilseed, rape, soybean, rice, barley, grass, palm, sesame, peanut, sunflower, tobacco, transgenic plant prep., particle bombardment, Agrobacterium tumefaciens vector-mediated gene expression, antisense oligonucleotide, transgenic mouse prep., DNA probe, monoclonal antibody prep., hybridoma, appl., oil degradation, bioremediation enzyme bioinformatics transgenic animal plant cereal grass Zea mays Solanum tuberosum fruit Lycopersicon esculentum Triticum aestivum oilseed Brassica napus legume Glycine max Oryza sativa Hordeum vulgare Sesamum indicum Arachis hypogaea Helianthus annuus Nicotiana tabacum bacterium hybridization cell culture pollutant degradation (24, 51)

SECTION: BIOMANUFACTURING and BIOCATALYSIS-Biocatalyst Application-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis;

GENETIC TECHNIQUES and APPLICATIONS-Transgenic Animals and Animal Models-BIOINFORMATICS and ANALYSIS-Hardware; BIOINFORMATICS and ANALYSIS-Software-BIOMANUFACTURING and BIOCATALYSIS-Animal/Plant Cell Culture; BIOMANUFACTURING and BIOCATALYSIS-Biocatalyst Isolation and Characterization-WASTE-DISPOSAL AND BIOREMEDIATION-Environmental Biotechnology; PHARMACEUTICALS-Antibodies-AGRICULTURAL BIOTECHNOLOGY-Plant Genetic Engineering

...ABSTRACT: 16) a method of inhibiting the expression of a phospholipase in a cell; (17) a protein preparation comprising the polypeptide, where the protein preparation comprises a liquid, a solid or a gel; (18) a heterodimer comprising the polypeptide...

... method for ameliorating or preventing lipopolysaccharide (LPS)-mediated toxicity; (58) a method for detoxifying an endotoxin ; (59) a method for deacylating a 2' or a 3 ' fatty acid chain from a...

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... a phospholipase D1 or a phospholipase D2 activity. The phospholipase activity comprises hydrolysis of a glycoprotein . The glycoprotein comprises a potato tuber. The phospholipase activity comprises a patatin enzymatic activity, or lipid acyl...

... comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector comprises an adenovirus vector, a retroviral vector or...

... associated viral vector. The cloning vehicle comprises a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC...

... providing the polypeptide; providing a phospholipase substrate; and contacting the polypeptide with the substrate and detecting a decrease in the amount of substrate or an increase in the amount of a ...

...the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a phospholipase activity. Identifying a phospholipase substrate comprises providing the polypeptide; providing a test substrate; and contacting the polypeptide with the test substrate and detecting a decrease in the amount of substrate or an increase in the amount of reaction...

... modulates the phospholipase activity. The phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of ...

7/9,K/6 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0376837 DBR Accession No.: 2005-22543 PATENT
For the detection of endotoxins, in a sample, the sample is incubated with bacteriophage tail proteins where the endotoxins bond to the tail proteins for detection - phage tail protein sample incubation for endotoxin detection and gene therapy
AUTHOR: MEYER R; SCHUETZ M; GRALLERT H; GRASSL R; MILLER S
PATENT ASSIGNEE: PROFOS AG 2005
PATENT NUMBER: WO 200562051 PATENT DATE: 20050707 WPI ACCESSION NO.: 2005-522260 (200553)
PRIORITY APPLIC. NO.: DE 1060844 APPLIC. DATE: 20031220
NATIONAL APPLIC. NO.: WO 2004DE2778 APPLIC. DATE: 20041220
LANGUAGE: German
ABSTRACT: DERWENT ABSTRACT: NOVELTY - For the detection of endotoxins in a sample, the sample is incubated with bacteriophage tail proteins. The endotoxin , bonded to the tail protein , is detected by enzyme linked immunoabsorbant assay (ELISA), chemical or enzyme detection reactions or split endotoxin components or capacity measurement. USE - The technique is for the detection of endotoxins which could have an adverse effect on genetically produced pharmaceuticals, genetic therapeutics, or substances injected into humans and animals. Endotoxins can also affect research materials in transfection experiments with mammalian cells. ADVANTAGE - The technique gives a simple and standardized method for the detection of endotoxins in solutions and samples.(63 pages)
DESCRIPTORS: phage tail protein sample incubation, ELISA, chemical, enzyme detection reaction, split endotoxin component, capacity measurement, appl. endotoxin det., human, animal gene therapy, genetically produced pharmaceutical, injected substance adverse effecte evaluation, transfection experiment analysis immunoassay toxin protein mammal (24, 36)
SECTION: THERAPEUTICS-Gene Therapy-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis

For the detection of endotoxins, in a sample, the sample is incubated with bacteriophage tail proteins where the endotoxins bond to the tail proteins for detection - phage tail protein sample incubation for endotoxin detection and gene therapy
ABSTRACT: DERWENT ABSTRACT: NOVELTY - For the detection of endotoxins in a sample, the sample is incubated with bacteriophage tail proteins. The endotoxin , bonded to the tail protein , is detected by enzyme linked immunoabsorbant assay (ELISA), chemical or enzyme detection reactions or split endotoxin components or capacity measurement. USE - The technique is for the detection of endotoxins which could have an adverse effect on genetically produced pharmaceuticals, genetic therapeutics, or...

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DESCRIPTORS: phage tail protein sample incubation, ELISA, chemical, enzyme detection reaction, split endotoxin component, capacity measurement, appl. endotoxin det., human, animal gene therapy,

genetically produced pharmaceutical, injected substance adverse effecte evaluation, transfection experiment analysis immunoassay toxin protein mammal (24, 36)

7/9,K/7 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0333474 DBR Accession No.: 2004-05766 PATENT
Method for detecting and removing endotoxins, useful for treating e.g. recombinantly produced pharmaceuticals or nucleic acid, by incubation with phage tail protein - involving vector-mediated gene transfer and expression in host cell for use in gene therapy
AUTHOR: SCHUETZ M; MEYER R; GRALLERT H; MILLER S
PATENT ASSIGNEE: PROFOS AG 2003
PATENT NUMBER: WO 200401418 PATENT DATE: 20031231 WPI ACCESSION NO.: 2004-071780 (200407)
PRIORITY APPLIC. NO.: DE 1007793 APPLIC. DATE: 20030224
NATIONAL APPLIC. NO.: WO 2003DE2096 APPLIC. DATE: 20030624
LANGUAGE: German
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Method for detecting endotoxins (I) comprises incubating a sample with a bacteriophage tail protein (II), then detecting any (I) bound to (II). DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method of removing (I) from a sample by incubation or contact with (II) that is immobilized, non-specifically or in a targeted manner, on a solid carrier. WIDER DISCLOSURE - This describes (1) phage proteins coupled at either end, especially the C-terminus, to a tag, particularly one that has a surface-exposed Cys residue for targeted biotinylation, and (2) nucleic acid encoding the proteins of (1). USE - The method is used to detect endotoxin (I) in e.g. recombinantly produced pharmaceuticals, gene therapy agents or materials intended for injection, also in research materials (nucleic acids) used in transfection experiments. When (II) is immobilized on a solid carrier, then method can be used for removing (I). ADVANTAGE - The method is applicable to all aqueous solutions; is superior to known detection and purification methods, and avoids the difficult preparation of antibodies directed against the core oligosaccharide of lipopolysaccharide. EXAMPLE - The p12 phage tail protein was reacted with sulfo-N-hydroxysuccinimide-LC-LC-biotin, then incubated with streptavidin-loaded chromatography material. A solution (1 ml) of bovine serum albumen (BSA) containing 10 EU/ml of endotoxin was stirred for 1 hour at room temperature with the p12-containing material (50 microlitres), then centrifuged and endotoxin concentration in the supernatant measured. Removal of endotoxin was 86% and recovery of albumen 90%.(41 pages)
DESCRIPTORS: recombinant phage tail protein prep., isol., vector-mediated gene transfer, expression in host cell, appl. gene therapy, pharmaceutical ind. (23, 11)
SECTION: THERAPEUTICS- Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; PHARMACEUTICALS-Other Pharmaceuticals-THERAPEUTICS-Gene Therapy
Method for detecting and removing endotoxins, useful for treating e.g. recombinantly produced pharmaceuticals or nucleic acid, by incubation with phage tail protein - involving vector-mediated gene transfer and expression in host cell for use in gene therapy
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Method for detecting endotoxins (I)

comprises incubating a sample with a bacteriophage tail protein (II), then detecting any (I) bound to (II). DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a...

... and (2) nucleic acid encoding the proteins of (1). USE - The method is used to detect endotoxin (I) in e.g. recombinantly produced pharmaceuticals, gene therapy agents or materials intended for injection...

... removing (I). ADVANTAGE - The method is applicable to all aqueous solutions; is superior to known detection and purification methods, and avoids the difficult preparation of antibodies directed against the core oligosaccharide of lipopolysaccharide. EXAMPLE - The p12 phage tail protein was reacted with sulfo-N-hydroxysuccinimide-LC-LC-biotin , then incubated with streptavidin-loaded chromatography material. A solution (1 ml) of bovine serum albumen (BSA) containing 10 EU/ml of endotoxin was stirred for 1 hour at room temperature with the p12-containing material (50 microlitres), then centrifuged and endotoxin concentration in the supernatant measured. Removal of endotoxin was 86% and recovery of albumen 90%.(41 pages)
DESCRIPTORS: recombinant phage tail protein prep., isol., vector-mediated gene transfer, expression in host cell, appl. gene therapy, pharmaceutical ind...
SECTION: THERAPEUTICS- Protein Therapeutics...
? t s8/9,k/1-9

8/9,K/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06035633 BIOSIS NO.: 198070067120
BACTERIO PHAGE T-4D RECEPTOR AND THE ESCHERICHIA-COLI CELL WALL STRUCTURE BINDING OF ENDO TOXIN-LIKE PARTICLES TO THE CELL WALL
AUTHOR: ZORZOPULOS J (Reprint); DELONG S; CHAPMAN V; KOZLOFF L M
AUTHOR ADDRESS: DEP MICROBIOL IMMUNOL, UNIV COLO HEALTH SCI CENT, DENVER, COLO 80262, USA**USA
JOURNAL: Journal of Bacteriology 142 (3): p982-991 1980
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A variety of degradative treatments were used to investigate the nature of the structure and components of the cell walls of E. coli B. The binding and localization of the endotoxin -like particles found on the cell walls were of special interest because some of them are associated with the site where the inner tail tube of bacteriophage T4D penetrates the cell wall. Modified cell walls were obtained by heating a suspension of bacterial cells originally in 0.1 M phosphate, pH 7.0, after the addition of 12.5 M NaOH to a final concentration of 0.25 M. With regard to the endotoxin -like particles, it was found that: at least part of them still remained bound to the modified cell wall after the alkali treatment; the subsequent incubation of alkali-treated cell walls with lysozyme destroyed the bacterial form and released a complex of endotoxin -like particles together with a fibrous material; treatment with 45% phenol at 70.degree. C removed the endotoxin -like particles from the surface of the alkali-treated cell walls, but most of the

fibrous material was left on the cell wall; and incubation of alkali-treated cell walls with 5 mM EDTA at 20.degree. C also removed the endotoxin -like particles, but did not disrupt the rodlike bacterial form. If the EDTA treatment was performed at 55.degree. C, the bacterium-like form was destroyed. These differential sensitivities to EDTA suggested that loosely bound divalent metal ions normally hold these endotoxin -like particles on the cell wall surface, but that probably more tightly bound metal ions are involved in the determination of cell shape. Analysis of the protein components of the alkali-treated cell walls showed that only 1 protein was present in significant amounts, and this protein had an electrophoretic mobility similar to that of the Braun lipoprotein. This protein was released from the alkali-treated cell walls upon heating with 2% sodium dodecyl sulfate at 100.degree. C. Phospholipids were also absent from this structure. The distribution of the remaining cell wall components on the alkali-treated cell walls is discussed.

REGISTRY NUMBERS: 9001-63-2: LYSOZYME
DESCRIPTORS: BRAUNS LIPO PROTEIN PROTEIN DIVALENT METAL ION LYSOZYME
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Microbiology; Physiology; Toxicology
BIOSYSTEMATIC NAMES: Viruses--Microorganisms; Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
COMMON TAXONOMIC TERMS: Viruses; Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: LYSOZYME
CONCEPT CODES:
10010 Comparative biochemistry
10054 Biochemistry methods - Proteins, peptides and amino acids
10056 Biochemistry methods - Lipids
10058 Biochemistry methods - Carbohydrates
10060 Biochemistry studies - General
10064 Biochemistry studies - Proteins, peptides and amino acids
10066 Biochemistry studies - Lipids
10068 Biochemistry studies - Carbohydrates
10069 Biochemistry studies - Minerals
10504 Biophysics - Methods and techniques
10614 External effects - Temperature as a primary variable
10618 External effects - Temperature as a primary variable - hot
10804 Enzymes - Methods
12100 Movement
22501 Toxicology - General and methods
23001 Temperature - General measurement and methods
31000 Physiology and biochemistry of bacteria
32000 Microbiological apparatus, methods and media
33504 Virology - Bacteriophage
BIOSYSTEMATIC CODES:
03000 Viruses
06702 Enterobacteriaceae

...ABSTRACT: components of the cell walls of E. coli B. The binding and localization of the endotoxin -like particles found on the cell walls were of special interest because some of them are associated with the site where the inner tail tube of bacteriophage T4D penetrates the cell wall. Modified cell walls were obtained by heating a suspension of...

...5 M NaOH to a final concentration of 0.25 M. With regard to the endotoxin -like particles, it was found that: at least part of them still remained bound to...
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...incubation of alkali-treated cell walls with 5 mM EDTA at 20.degree. C also removed the endotoxin -like particles, but did not disrupt the rodlike bacterial form. If the EDTA treatment was...

...These differential sensitivities to EDTA suggested that loosely bound divalent metal ions normally hold these endotoxin -like particles on the cell wall surface, but that probably more tightly bound metal ions are involved in the determination of cell shape. Analysis of the protein components of the alkali-treated cell walls showed that only 1 protein was present in significant amounts, and this protein had an electrophoretic mobility similar to that of the Braun lipoprotein. This protein was released from the alkali-treated cell walls upon heating with 2% sodium dodecyl sulfate...
DESCRIPTORS: BRAUNS LIPO PROTEIN PROTEIN DIVALENT METAL ION LYSOZYME

8/9,K/2 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

12951668 Genuine Article#: 836BM Number of References: 30
Title: Preparation of endotoxin -free bacteriophages
Author(s): Boratynski J (REPRINT) ; Syper D; Weber-Dabrowska B; Lusiak-Szelachowska M; Pozniak G; Gorski A
Corporate Source: Polish Acad Sci,Lab Biomed Chem, Dept Expt Oncol,PL-53114 Wroclaw//Poland/ (REPRINT); Polish Acad Sci,Lab Biomed Chem, Dept Expt Oncol,PL-53114 Wroclaw//Poland/; Polish Acad Sci,Inst Immunol & Expt Therapy, Lab Bacteriophages,PL-53114 Wroclaw//Poland/; Med Acad Warsaw,Inst Transplantat,PL-02006 Warsaw//Poland/; Wroclaw Tech Univ,Inst Organ & Polymer Technol,PL-02006 Warsaw//Poland/(borat@iitd.pan.wroc.pl)
Journal: CELLULAR & MOLECULAR BIOLOGY LETTERS, 2004, V9, N2, P253-259
ISSN: 1425-8153 Publication date: 20040000
Publisher: CELLULAR & MOLECULAR BIOLOGY LETTERS, UNIV WROCLAW, INST BIOCHEM, DEPT GENETIC BIOCHEMISTRY, PRZBYSZEWSKIEGO 63/77, 51-148 WROCLAW, POLAND
Language: English Document Type: ARTICLE
Geographic Location: Poland
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY; CELL BIOLOGY
Abstract: Bacteriophages (phages) are bacterial viruses that interact with bacterial walls and invade bacterial cells. Moreover, they disturb bacterial metabolism and lead to bacteria lysis. In the case of Gram-negative bacteria crude phage cultures, apart from the phages themselves, the bacterial debris, bacterial proteins and nucleic acids contain endotoxins. These endotoxins (lipopolysaccharides) posses a high degree of toxicity in vitro and in vivo, and their removal is essential for safety in antibacterial bacteriophage therapy. An effective, scaleable purification of bacteriophages from endotoxins was

accomplished by sequential ultrafiltration through polysulfone membrane (30 nm) followed by chromatography on sepharose 4B and Matrex Cellulofine Sulfate. The phage fraction after gel filtration chromatography routinely contained endotoxins in the 150-2500 EU/ml range. The procedure yielded bacteriophages contaminated with as little as 0.4-7 EU/ml (Limulus assay). This value lies within the permitted level for intravenous applications (5 EU/kg/h by European Pharmacopoeia, 1997).

Descriptors--Author Keywords: endotoxin ; LPS ; phage ; bacteriophage purification ; therapy

Identifiers--KeyWord Plus(R): EFFICIENT REMOVAL ; PROTEIN SOLUTIONS; INNATE IMMUNITY; BACTERIAL; LIPOPOLYSACCHARIDES; PURIFICATION; FIBER

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Title: Preparation of endotoxin -free bacteriophages

...Abstract: endotoxins (lipopolysaccharides) posses a high degree of toxicity in vitro and in vivo, and their removal is essential for safety in antibacterial lbacteriophage therapy. An effective, scaleable purification of bacteriophages from endotoxins was accomplished by sequential ultrafiltration through...

...Identifiers--EFFICIENT REMOVAL ; PROTEIN SOLUTIONS; INNATE IMMUNITY; BACTERIAL; LIPOPOLYSACCHARIDES; PURIFICATION; FIBER

8/9,K/3 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

12705892 EMBASE No: 2004297828
Preparation of endotoxin -free bacteriophages
Boratynski J.; Syper D.; Weber-Da(cedil)browska B.; L(stroke)usiak-Szelachowska M.; Pozniak G.; Gorski A.
J. Boratynski, Department of Experimental Oncology, Inst. of Immunol. and Exp. Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wrocl(stroke)aw Poland
AUTHOR EMAIL: borat@iitd.pan.wroc.pl
Cellular and Molecular Biology Letters (CELL. MOL. BIOL. LETT.) (Poland) 2004, 9/2 (253-259)
CODEN: CMBLF ISSN: 1425-8153
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 30

Bacteriophages (phages) are bacterial viruses that interact with bacterial walls and invade bacterial cells. Moreover, they disturb bacterial metabolism and lead to bacteria lysis. In the case of Gram-negative bacteria crude phage cultures, apart from the phages themselves, the bacterial debris, bacterial proteins and nucleic acids contain endotoxins. These endotoxins (lipopolysaccharides) posses a high degree of toxicity in vitro and in vivo, and their removal is essential for safety in antibacterial bacteriophage therapy. An effective, scaleable purification of bacteriophages from endotoxins was accomplished by sequential ultrafiltration through polysulfone membrane (30 nm) followed by chromatography on sepharose 4B and Matrex Cellulofine Sulfate. The phage fraction after gel filtration chromatography routinely contained endotoxins in the 150-2500 EU/ml range. The procedure yielded bacteriophages contaminated with as little as 0.4-7 EU/ml (Limulus assay). This value lies within the permitted level for intravenous applications (5 EU/kg/h by European Pharmacopoeia, 1997).

DRUG DESCRIPTORS:
* endotoxin
bacterial protein ; nucleic acid; bacterium lipopolysaccharide; polysulfone; bacterium lysate
MEDICAL DESCRIPTORS:
* bacteriophage
bacterial membrane; bacterial cell; bacterial metabolism; bacteriolysis; Gram negative bacterium; bacterium culture; purification; ultrafiltration; gel filtration chromatography; nonhuman; article
CAS REGISTRY NO.: 25135-51-7 (polysulfone); 88402-38-4 (bacterium lysate)
SECTION HEADINGS:
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology

Preparation of endotoxin -free bacteriophages

...endotoxins (lipopolysaccharides) posses a high degree of toxicity in vitro and in vivo, and their removal is essential for safety in antibacterial bacteriophage therapy. An effective, scaleable purification of bacteriophages from endotoxins was accomplished by sequential ultrafiltration through...

DRUG DESCRIPTORS:
* endotoxin
bacterial protein ; nucleic acid; bacterium lipopolysaccharide; polysulfone; bacterium lysate

MEDICAL DESCRIPTORS:
* bacteriophage

8/9,K/4 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

02019394 EMBASE No: 1981006563
Bacteriophage T4D receptor and the Escherichia coli cell wall structure: binding of endotoxin -like particles to the cell wall
Zorzopulos J.; DeLong S.; Chapman V.; Kozloff L.M.
Dept. Microbiol. Immunol., Univ. Colorado Hlth Sci. Cent., Denver, Colo. 80262 United States
Journal of Bacteriology (J. BACTERIOL.) (United States) 1980, 142/3 (982-991)
CODEN: JOBAA
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

A variety of degradative treatments have been used to investigate the nature of the structure and components of the cell walls of Escherichia coli B. The binding and localization of the endotoxin -like particles found on the cell walls were of special interest because some of them are associated with the site where the inner tail tube of bacteriophage T4D penetrates the cell wall. Modified cell walls were obtained by heating a suspension of bacterial cells originally in 0.1 M phosphate, pH 7.0, after the addition of 12.5 M NaOH to a final concentration of 0.25 M. With regard to the endotoxin -like particles, it was found that: at least part of them still remained bound to the modified cell wall after the alkali treatment; the subsequent incubation of alkali-treated cell walls with lysozyme destroyed the bacterial form and released a complex of endotoxin -like particles together with a fibrous material; on the other hand, treatment with 45% phenol at 70degreeC removed the endotoxin -like particles from the surface of the alkali-treated cell walls, but most of the fibrous material was left on the cell wall; and (iv) incubation of alkali-treated cell walls with 5 mM ethylenediaminetetraacetic acid at 20degreeC also removed the endotoxin -like particles, but did not disrupt the rodlike bacterial form. However, if the ethylenediaminetetraacetic acid treatment was performed at 55degreeC, the bacterium-like form was destroyed. These differential sensitivities to ethylenediaminetetraacetic acid suggested that loosely bound divalent metal ions normally hold these endotoxin -like particles on the cell wall surface, but that probably more tightly bound metal ions are involved in the determination of cell shape. Analysis of the protein components of the alkali-treated cell walls showed that only one protein was present in significant amounts, and this protein had an electrophoretic mobility similar to that of the Braun lipoprotein. This protein was released from the alkali-treated cell walls upon heating with 2% sodium dodecyl sulfate at 100degreeC. Phospholipids were also absent from this structure. The distribution of the remaining cell wall components on the alkali-treated cell walls is discussed.

DRUG DESCRIPTORS:
* bacteriophage receptor
MEDICAL DESCRIPTORS:
* bacteriophage ; *bacterial cell wall; *escherichia coli
virus cell interaction; in vitro study; animal experiment

SECTION HEADINGS:
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
047 Virology

Bacteriophage T4D receptor and the Escherichia coli cell wall structure: binding of endotoxin -like particles to the cell wall

...components of the cell walls of Escherichia coli B. The binding and localization of the endotoxin -like particles found on the cell walls were of special interest because some of them are associated with the site where the inner tail tube of bacteriophage T4D penetrates the cell wall. Modified cell walls were obtained by heating a suspension of...

...5 M NaOH to a final concentration of 0.25 M. With regard to the endotoxin -like particles, it was found that: at least part of them still remained bound to...
...alkali-treated cell walls with lysozyme destroyed the bacterial form and released a complex of endotoxin -like particles together with a fibrous material; on the other hand, treatment with 45% phenol at 70degreeC removed the endotoxin -like particles from the surface of the alkali-treated cell walls, but most of the...

...iv) incubation of alkali-treated cell walls with 5 mM ethylenediaminetetraacetic acid at 20degreeC also removed the endotoxin -like particles, but did not disrupt the rodlike bacterial form. However, if the ethylenediaminetetraacetic acid...

...differential sensitivities to ethylenediaminetetraacetic acid suggested that loosely bound divalent metal ions normally hold these endotoxin -like particles on the cell wall surface, but that probably more tightly bound metal ions are involved in the determination of cell shape. Analysis of the protein components of the alkali-treated cell walls showed that only one protein was present in significant amounts, and this protein had an electrophoretic mobility similar to that of the Braun lipoprotein. This protein was released from the alkali-treated cell walls upon heating with 2% sodium dodecyl sulfate...
DRUG DESCRIPTORS:
* bacteriophage receptor
MEDICAL DESCRIPTORS:
* bacteriophage ; *bacterial cell wall; *escherichia coli

8/9,K/5 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

15535047 PMID: 16007984
Performance of a membrane adsorber for trace impurity removal in biotechnology manufacturing.
Phillips Michael; Cormier Jason; Ferrence Jennifer; Dowd Chris; Kiss Robert; Lutz Herbert; Carter Jeffrey
Biopharmaceutical R&D Division, Millipore Corp., 32 Wiggins Ave, Bedford, MA 01730, USA.
Journal of chromatography. A (Netherlands) Jun 17 2005, 1078 (1-2) p74-82, ISSN 0021-9673--Print Journal Code: 9318488
Publishing Model Print
Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Membrane adsorbers provide an attractive alternative to traditional bead-based chromatography columns used to remove trace impurities in downstream applications. A linearly scalable novel membrane adsorber family designed for the efficient removal of trace impurities from biotherapeutics, are capable of reproducibly achieving greater than 4 log removal of mammalian viruses, 3 log removal of endotoxin and DNA, and greater than 1 log removal of host cell protein . Single use, disposable membrane adsorbers eliminate the need for costly and time consuming column packing and cleaning validation associated with bead-based chromatography systems, and minimize the required number and volume of buffers. A membrane adsorber step reduces process time, floor space, buffer usage, labor cost, and improves manufacturing flexibility. This "process compression" effect is commonly associated with reducing the number of processing steps. The rigid microporous structure of the membrane layers allows for high process flux operation and uniform bed consistency at all processing scales.

Descriptors: *Bacteriophages--isolation and purification--IP; *Chromatography, Ion Exchange--methods--MT; *DNA --isolation and purification--IP; *Endotoxins--isolation and purification--IP; *Membranes, Artificial; Adsorption; Animals; Antibodies, Monoclonal; Bacterial Proteins --isolation and purification--IP; Bacteriophage phi 6--isolation and purification--IP; Bacteriophage phi X 174--isolation and purification--IP ; Biotechnology--methods--MT; Chromatography, Ion Exchange--instrumentation --IS; Escherichia coli--virology--VI; Humans; Hydrogen-Ion Concentration; Leukemia Virus, Murine--isolation and purification--IP; Mice; Mice Minute Virus--isolation and purification--IP; Osmolar Concentration; Pilot Projects; Pseudomonas pseudoalcaligenes--virology--VI; Reproducibility of Results; Simian virus 40--isolation and purification--IP

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Bacterial Proteins); 0 (Endotoxins); 9007-49-2 (DNA)

Record Date Created: 20050712
Record Date Completed: 20050908

Performance of a membrane adsorber for trace impurity removal in biotechnology manufacturing.

Membrane adsorbers provide an attractive alternative to traditional bead-based chromatography columns used to remove trace impurities in downstream applications. A linearly scalable novel membrane adsorber family designed for the efficient removal of trace impurities from biotherapeutics, are capable of reproducibly achieving greater than 4 log removal of mammalian viruses, 3 log removal of endotoxin and DNA, and greater than 1 log removal of host cell protein . Single use, disposable membrane adsorbers eliminate the need for costly and time consuming column packing...

; Adsorption; Animals; Antibodies, Monoclonal; Bacterial Proteins --isolation and purification--IP; Bacteriophage phi 6--isolation and purification--IP; Bacteriophage phi X 174--isolation and purification--IP ; Biotechnology--methods--MT; Chromatography, Ion Exchange--instrumentation --IS...

8/9,K/6 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0415596 DBR Accession No.: 2007-01534 PATENT
Novel nucleic acid encoding polypeptide having hydrolase activity, useful in food supplements, identifying hydrolase modulators, hydrolyzing triacylglycerol, preventing lipopolysaccharide-mediated toxicity, and preparation of propionic acid - transgenic plant, artificial chromosome, transgenic plant for recombinant lipase production for use in paper, pharmaceutical and food industry
AUTHOR: KEROVUO J S; MCCANN R; WEINER D; SOLBAK A I
PATENT ASSIGNEE: DIVERSA CORP 2006
PATENT NUMBER: WO 200696834 PATENT DATE: 20060914 WPI ACCESSION NO.: 2007-008852 (200701)
PRIORITY APPLIC. NO.: US 660122 APPLIC. DATE: 20050308
NATIONAL APPLIC. NO.: WO 2006US8555 APPLIC. DATE: 20060308
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A nucleic acid (NA) encoding a polypeptide having hydrolase activity, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) nucleic acid probe for identifying nucleic acid encoding polypeptide with hydrolase activity; (2) amplification primer pair amplifying SEQ ID No. 1-991 (odd SEQ ID numbers); (3) amplifying (M45) nucleic acid encoding polypeptide with hydrolase activity; (4) expression cassette, vector and cloning vehicle, having nucleic acid; (5) transformed cell having nucleic acid, or expression cassette having nucleic acid; (6) transgenic non-human animal/seed/plant, having the nucleic acid sequence; (7) an antisense oligonucleotide, having nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to (SEQ ID No. 1-991); (8) inhibiting (M46) translation of hydrolase message in cell; (9) a double-stranded inhibitory RNA molecule, having subsequence of nucleic acid; (10) inhibiting (M47) the expression of a hydrolase in a cell; (11) an isolated or recombinant polypeptide (P1); (12) isolated/recombinant polypeptide having (P1) and lacking a signal sequence or having heterologous signal sequence; (13) protein preparation having (P1), where the preparation is liquid/solid/gel; (14) heterodimer having (P1) and second domain; (15) immobilized polypeptide having (P1); (16) array having an immobilized polypeptide or immobilized nucleic acid; (17) an isolated or recombinant antibody that specifically binds to (P1); (18) hybridoma having an antibody that binds to (P1); (19) food supplement for an animal and edible enzyme delivery matrix having (P1); (20) isolating or identifying (M1) a polypeptide with a hydrolase activity; (21) producing (M2) an anti-hydrolase antibody; (22) producing (M3) a recombinant polypeptide; (23) identifying a polypeptide (M4) having a hydrolase activity or hydrolase substrate; (24) determining (M5) whether a test compound specifically binds to a polypeptide; (25) identifying (M6) a modulator of a hydrolase activity; (26) a computer system having a processor and a data storage device, where the data storage device has stored on it (P1); (27) identifying (M7) a feature in a sequence; (28) comparing (M8) two sequences; (29) isolating a nucleic acid encoding a polypeptide with a hydrolase activity from an environmental sample; (30) generating (M9) a variant of a nucleic acid encoding a hydrolase; (31) modifying (M10) codons in a nucleic acid encoding a hydrolase to increase its expression in a host cell; (32) producing (M11) library of nucleic acids encoding several modified hydrolase active sites/substrate binding sites; (33) making (M12) small molecule; (34) modifying (M13) small molecule; (35) determining (M14) functional fragment of hydrolase enzyme; (36) whole cell engineering

(M15) of new/modified phenotypes using real-time metabolic flux analysis; (37) hydrolyzing (M16) triacylglycerol (TAG), diacylglycerol (DAG) or monoacylglycerol (MAG); (38) removing /decreasing (M17) amount of TAG, DAG or MAG from composition; (39) increasing (M18) thermotolerance/thermostability of hydrolase polypeptide; (40) overexpressing (M19) recombinant hydrolase polypeptide in cell; (41) detergent composition having (P1) or polypeptide encoded by the nucleic acid; (42) washing (M20) object; (43) hydrolyzing (M21) oil in feed or food prior to consumption by animal; (44) a feed having (P1) or polypeptide encoded by the nucleic acid; (45) a composition having an oil and the recombinant polypeptide; (46) pharmaceutical containing recombinant polypeptide; (47) making (M22) a transgenic plant; (48) expressing (M23) heterologous nucleic acid sequence in plant cell; (49) signal sequence having a peptide having a subsequence of (P1); (50) chimeric protein having a first domain having the signal sequence and second domain; (51) biocatalytic (M24) synthesis of a structured lipid; (52) preparation (M25) of an optical isomer of a propionic acid from a racemic ester of the propionic acid; (53) stereoselectively (M26) hydrolyzing racemic mixtures of esters of 2-substituted acids; (54) oil or fat modification (M27); (55) hydrolysis (M28) of polyunsaturated fatty acid (PUFA) esters; (56) selective (M29) hydrolysis of PUFA esters over saturated fatty acid esters; (57) preparing (M30) a food or a feed additive having PUFA; (58) treatment (M31) method of latex; (59) refining (M32) a lubricant; (60) treating (M33) a fabric; (61) decreasing (M34) the amount of a food or oil stain; (62) dietary composition having hydrolase; (63) reducing (M35) fat content in milk or vegetable-based dietary compositions; (64) dietary composition; (65) catalyzing (M36) an interesterification reaction to produce new triglycerides; (66) transesterification (M37) method for preparing a margarine oil having a low trans-acid and a low intermediate chain fatty acid content; (67) ameliorating/preventing (M38) lipopolysaccharide- mediated toxicity; (68) detoxifying (M39) an endotoxin ; (69) deacylating (M40) 2' or 3' fatty acid chain from a lipid A; (70) hydrolyzing (M41) composition having a cellulose or lipophilic compound; (71) making (M42) paper; (72) composition which encoded by nucleic acid; (73) generating (M43) sterol/glycerol/free fatty acid by hydrolyzing composition having cellulose/lipophilic compound; (74) decreasing (M44) amount of lipophilic extract in compound having cellulose; and (75) fabric/yarn/fiber having hydrolase.

BIOTECHNOLOGY - Preparation (claimed): The nucleic acid is isolated or recovered from an environmental sample, by providing the amplification primer sequence pair, isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair, and combining the nucleic acid with the primer pair and amplifying nucleic acid from the environmental sample or providing a polynucleotide probe comprising the nucleic acid sequence, isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe, combining the isolated nucleic acid or the treated environmental sample with the polynucleotide probe and isolating a nucleic acid that specifically hybridizes with the polynucleotide probe. The environmental sample comprises water sample, liquid sample, soil sample, air sample or biological sample. The biological sample is derived from bacterial cell, protozoan cell, insect cell, yeast cell, plant cell, fungal cell or mammalian cell. Preferred Nucleic Acid: NA is an isolated or

recombinant nucleic acid (a) having at least 51-99% sequence identity to SEQ ID No. 1-991 (odd SEQ ID numbers) over region of 50, 75, 100, 150-1150 (in multiples of 50) or more residues, polypeptide/peptide generating antibody that binds to polypeptide having SEQ ID No. 2-992, and sequence identities are determined by analysis with sequence comparison algorithm or by visual inspection, nucleic acid sequence that hybridizes under stringent conditions to SEQ ID No. 1-991 (odd SEQ ID numbers), and nucleic acid is 20-1000 (in multiples of 10) or more residues in length or full length of gene or transcript, or nucleic acid sequence complementary to it, or (b) sequence that hybridizes to a nucleic acid having SEQ ID NO. 1-991 (odd SEQ ID numbers), where the nucleic acid encodes a polypeptide having a hydrolase activity. The sequence identity is over a region of at least 51-99% or more or is 100% or over a region of at least 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550 or more residues, or the full length of a gene or transcript, preferably 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900,1000 or more residues in length. The nucleic acid sequence comprises a sequence having SEQ ID Nos. 1-991 (odd SEQ ID numbers) or encodes a polypeptide having SEQ ID No. 2-992 (even SEQ ID numbers). The sequence comparison algorithm is a BLAST version 2.2.2 algorithm, where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default. The hydrolase activity includes lipase activity, protease activity, esterase activity or phospholipase activity. The lipase activity involves hydrolyzing a triacylglycerol to a diacylglycerol and a free fatty acid or hydrolyzing a triacylglycerol to a monoacylglycerol and free fatty acids or hydrolyzing a diacylglycerol to a monoacylglycerol and free fatty acids, or hydrolyzing monoacylglycerol to free fatty acid and glycerol, or hydrolyzing triacylglycerol, diacylglycerol or monoacylglycerol or synthesizing a tryacylglycerol from diacylglycerol or monoacylglycerol and free fatty acids. The lipase activity involves synthesizing 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1,3- distearoyl-2-oleoylglycerol (SOS), 1-palmitoyl-2-oleoyl-3-stearoyl glycerol (POS) or 1-oleoyl-2,3-dimyristoylglycerol (OMM), long chain polyunsaturated fatty acids, arachidonic acid, docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA). The lipase activity is triacylglycerol, diacylglycerol or monoacylglycerol position specific. The lipase activity is Sn2-specific, Sn1- or Sn3-specific or fatty acid specific. The lipase activity comprises modifying oils by hydrolysis, alcoholysis, esterification, transesterification or interesterification. The lipase activity is regio-specific or chemoselective. The lipase activity comprises synthesis of enantiomerically pure chiral products. The lipase activity comprises synthesis of umbelliferyl fatty acid (FA) esters. The lipase activity is thermostable. The polypeptide retains a lipase activity under conditions comprising a temperature range of 37-95degreesC, 55-85degreesC, 70-95degreesC, or 90-95degreesC. The hydrolase activity is thermotolerant. The polypeptide retains a hydrolase activity after exposure to a temperature in the range from greater than 37-95degreesC, 55-85degreesC, or 90-95degreesC. The stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of 65degreesC for 15 minutes. Preferred Polypeptide: P1 has at least 50-99% or more identity to SEQ ID No. 2-992 (even SEQ ID numbers), over a region of at least 20-700 (in multiples of 10) or more residues, where optinally the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual

inspection, or encoded by the nucleic acid; Preferred Probe: The probe comprises 10 consecutive bases of SEQ ID No. 1-991 (odd SEQ ID numbers), where the probe identifies the nucleic acid by binding or hybridization and the sequence identities are determined by analysis with sequence comparison algorithm or by visual inspection Preferred Cloning Vehicle: The cloning vehicle comprises viral vector, plasmid, phage, phagemid, cosmid, fosmid, bacteriophage or artificial chromosome. The viral vector comprises adenovirus vector, retroviral vector or adeno-associated viral vector or comprises bacterial artificial chromosome (BAC), plasmid, bacteriophage P1-derived vector (PAC), yeast artificial chromosome (YAC), or mammalian artificial chromosome (MAC). Preferred Host Cell: The cell is bacterial cell, mammalian cell, fungal cell, yeast cell, insect cell or plant cell, preferably potato, rice, corn, wheat, tobacco or barley cell. Preferred Transgenic Animal: The animal is a mouse. Preferred Transgenic Plant: The plant is corn plant, sorghum plant, potato plant, tomato plant, wheat plant, oilseed plant, rapeseed plant, soybean plant, rice plant, barley plant, grass, or tobacco plant. Preferred Transgenic Seed: The seed is rice, corn seed, wheat kernel, oilseed, rapeseed, soybean seed, palm kernel, sunflower seed, sesame seed, rice, barley, peanut or tobacco plant seed. Preferred Heterodimer: The second domain is a polypeptide and the heterodimer is a fusion protein or epitope or tag. Preferred Matrix: The delivery matrix comprises a pellet. Preferred Method: Isolating or identifying (M1) a polypeptide with a hydrolase activity, involves providing isolated or recombinant antibody, sample comprising polypeptides, and contacting the sample with the antibody under conditions, where the antibody can specifically bind to the polypeptide, thus isolating or identifying a polypeptide having a hydrolase activity. Producing (M2) anti-hydrolase antibody, involves administering to a non-human animal the nucleic acid or (P1) to generate a humoral immune response, thus making an anti-hydrolase antibody. Producing (M3) a recombinant polypeptide, involves providing the nucleic acid operably linked to a promoter, and expressing the nucleic acid under conditions that allow expression of the polypeptide. Identifying (M4) a polypeptide having a hydrolase activity or hydrolase substrate, involves providing a recombinant polypeptide, hydrolase substrate, and contacting the polypeptide with the substrate and detecting a decrease in the amount of substrate or increase in the amount of a reaction product, where decrease in the amount of the substrate or increase in the amount of the reaction product detects a polypeptide having a hydrolase activity or where a decrease in amount of substrate or increase in amount of reaction product identifies test substrate as a hydrolase substrate. The substrate is fatty acid, TAG, DAG or MAG. Determining (M5) whether a test compound specifically binds to a polypeptide, involves expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, where the nucleic acid has SEQ ID No. 1-991 (odd SEQ ID numbers), or providing (P1), and providing a test compound, contacting the polypeptide with the test compound and determining whether test compound specifically binds to polypeptide. Identifying (M6) a modulator of a hydrolase activity, involves providing the recombinant polypeptide, test compound, contacting the polypeptide with the test compound and measuring hydrolase activity, where a change in activity measured in presence of test compound compared to activity in absence provides a determination that the test compound modulates hydrolase activity. Identifying (M7) a feature in a sequence, involves reading the sequence using a computer program which

identifies one or more features in a sequence, where the sequence comprises (P1) or a nucleic acid sequence, a polypeptide encoded by the nucleic acid and identifying one or more features in the sequence with the computer program. Comparing (M8) a first sequence to a second sequence, involves reading the first sequence and the second sequence through use of a computer program which compares sequences, where the first sequence comprises a (P1) or a nucleic acid sequence, or a polypeptide encoded by the nucleic acid and determining differences between first and second sequence with the computer program. Generating (M9) a variant of a nucleic acid encoding a polypeptide with a hydrolase activity, involves providing a template nucleic acid comprising the nucleic acid sequence and modifying, deleting or adding one or more nucleotides in the template sequence, or their combination. Modifying (M10) codons in a nucleic acid encoding a polypeptide with a hydrolase activity to increase its expression in a host cell, involves providing a nucleic acid encoding a polypeptide with a hydrolase activity comprising the nucleic acid sequence and identifying a non-preferred or a less preferred codon in the nucleic acid and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, where a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thus modifying the nucleic acid to increase its expression in a host cell or identifying a codon in the nucleic acid and replacing it with a different codon encoding the same amino acid as the replaced codon, thus modifying codons in a nucleic acid encoding a hydrolase or identifying at least one preferred codon in the nucleic acid and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, where a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell. Producing (M11) a library of nucleic acids encoding a plurality of modified hydrolase active sites or substrate binding sites, where the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site, involves providing a first nucleic acid encoding a first active site or first substrate binding site, where the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to the nucleic acid sequence, and the nucleic acid encodes a hydrolase active site or a hydrolase substrate binding site, mutagenic oligonucleotides that encode naturally-occurring amino acid variants at several targeted codons in the first nucleic acid and using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized. The host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell. Making (M12) a small molecule, involves providing several biosynthetic enzymes capable of synthesizing or modifying a small molecule, where one of the enzymes comprises a hydrolase enzyme encoded by the nucleic acid, substrate for enzymes, and reacting the substrate with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions. Modifying (M13) a small molecule, involves providing a hydrolase enzyme, where the enzyme comprises the recombinant polypeptide, or a polypeptide encoded by the

nucleic acid sequence, small molecule and reacting the enzyme with the small molecule that facilitate enzymatic reaction catalyzed by the hydrolase enzyme. Determining (M14) a functional fragment of a hydrolase enzyme, involves providing a hydrolase enzyme comprising the recombinant polypeptide, or polypeptide encoded by the nucleic acid and deleting several amino acid residues and testing the remaining subsequence for a hydrolase activity. Method (M15) for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, involves making a modified cell by modifying the genetic composition of a cell, where the genetic composition is modified by addition to the cell of the nucleic acid, culturing the modified cell, measuring metabolic parameter of the cell by monitoring the cell culture in real time and analyzing the data to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thus identifying an engineered phenotype in the cell using real-time metabolic flux analysis. Hydrolyzing (M16) a triacylglycerol, diacyl glycerol or monoacylglycerol, involves providing the polypeptide having a hydrolase activity, or polypeptide encoded by the nucleic acid,composition comprising triacyl glycerol, diacyl glycerol or monoacylglycerol, and contacting the polypeptide with the composition, where the polypeptide hydrolyzes triacylglycerol, diacylglycerol or monoacylglycerol.

Removing (M17) or decreasing the amount of triacylglycerol, diacylglycerol or monoacylglycerol from a composition, involves providing the recombinant polypeptide having a hydrolase activity, or a polypeptide encoded by the nucleic acid, providing a composition comprising a triacylglycerol, diacylglycerol or monoacylglycerol, and contacting the polypeptide with the composition, where the polypeptide removes or decreases the amount of the triacylglycerol, diacylglycerol or monoacylglycerol. Increasing (M18) thermotolerance or thermostability of a hydrolase polypeptide, involves glycosylating a hydrolase polypeptide, where the polypeptide comprises at least thirty contiguous amino acids of (P1) or a polypeptide encoded by the nucleic acid. The hydrolase specific activity is thermostable or thermotolerant at a temperature in range greater than 37-95degreesC. Overexpressing (M19) a recombinant hydrolase polypeptide in a cell, involves expressing a vector comprising the nucleic acid sequence, where overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector. Washing (M20) an object, involves providing a composition comprising the recombinant polypeptide having a hydrolase activity, or a polypeptide encoded by the nucleic acid, providing an object, and contacting the polypeptide and the object under conditions where the composition can wash the object. Hydrolyzing (M21) an oil in a feed or a food prior to consumption by an animal, involves obtaining a feed material comprising an oil, where the oil can be hydrolyzed by the recombinant polypeptide having a hydrolase activity, or a polypeptide encoded by the nucleic acid, and adding the polypeptide to the feed or food material for a sufficient period to cause hydrolysis of the oil and formation of a treated food or feed. The food or feed comprises rice, corn, barley, wheat, legumes or potato. Making (M22) a transgenic plant, involves introducing a heterologous nucleic acid sequence into the cell, where the heterologous nucleic sequence comprises SEQ ID No. 1-991 (odd SEQ ID numbers) and producing a transgenic plant from the transformed cell. Expressing (M23) a heterologous nucleic acid sequence in a plant cell, involves transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, and growing the plant under

conditions, where the heterologous nucleic acids sequence is expressed in the plant cell. Biocatalytic (M24) synthesis of a structured lipid, involves providing the recombinant polypeptide having hydrolase and composition comprising triacylglyceride, contacting the polypeptide with the composition under conditions, where the polypeptide hydrolyzes acyl residue at the Sn2 position or Sn1 or Sn3 of triacylglyceride, thus producing a 1,3-diacylglyceride or 1,2-diacylglyceride or 2,3-diacylglyceride and promoting acyl migration under kinetically controlled conditions, providing an R1 ester, R1-specific hydrolase and contacting the 1,3-DAG with the R1 ester and the R1-specific hydrolase, where the R1-specific hydrolase catalyzes esterification of the Sn2 position, thus producing the structured lipid. the lipid includes cocoa butter alternative (CBA), synthetic cocoa butter, natural cocoa butter, 1,3- dipalmitoyl-2-oleoylglycerol (POP), 1,3-distearoyl-2-oleoylglycerol (SOS), 1-palmitoyl- 2-oleoyl-3-stearoylglycerol (POS) or 1-oleoyl-2,3-dimyristoylglycerol (OMM). Preparation (M25) of an optical isomer of a propionic acid from a racemic ester of the propionic acid, involves providing a hydrolase being stereoselective for an optical isomer of the propionic acid, racemic esters contacting the polypeptide with the racemic esters, where the polypeptide can selectively catalyze the hydrolysis of the esters, thus producing the optical isomer of the propionic acid. Stereoselectively (M26) hydrolyzing racemic mixtures of esters of 2-substituted acids, involves providing a hydrolase the stereoselective, composition comprising a racemic mixture of esters of 2- substituted acids and contacting the polypeptide with the composition under conditions, where the polypeptide can selectively hydrolyze the esters. Method (M27) for oil or fat modification, involves providing a hydrolase, oil or fat, and contacting the hydrolase with the oil or fat under conditions, where the hydrolase can modify the oil or fat. The oil comprises a glycerol ester of PUFA, or fish, animal or vegetable oil. Hydrolysis (M28) of polyunsaturated fatty acid (PUFA) esters, involves providing a hydrolase, composition comprising PUFA esters, and contacting the hydrolase with the composition under conditions, where the hydrolase can hydrolyze PUFA ester. Selective (M29) hydrolysis of PUFA esters over saturated fatty acid esters, involves providing a hydrolase having a lipase activity and selectively hydrolyzes PUFA esters, composition comprising a mixture of polyunsaturated and saturated esters, and contacting the polypeptide with the composition under conditions, where the polypeptide can selectively catalyze the hydrolysis of PUFA esters. Preparing (M30) a food or a feed additive comprising PUFA, involves providing a hydrolase that selectively hydrolyzes PUFA esters, composition comprising a PUFA ester, and contacting the polypeptide with the composition under conditions, where the polypeptide can selectively catalyze the hydrolysis of PUFA esters, thus producing the PUFA-containing food or feed additive. Treatment (M31) of latex, involves providing a hydrolase having selectivity for a saturated ester over an unsaturated ester, thus converting the saturated ester to its corresponding acid and alcohol, a latex composition comprising saturated and unsaturated esters contacting the hydrolase with the composition under conditions where the polypeptide can selectively hydrolyze saturated esters, thus treating the latex. Refining (M32) a lubricant, involves providing a composition comprising hydrolase and lubricant, treating the lubricant with the hydrolase under conditions, where the hydrolase can selective hydrolyze oils in the lubricant, thus refining it. Treating (M33) a fabric, involves providing a composition comprising hydrolase that selectively hydrolyze carboxylic esters, and

providing a fabric, treating the fabric with the hydrolase under condition, where the hydrolase can selectively hydrolyze carboxylic esters, thus treating the fabric. Removing (M34) or decreasing the amount of a food or oil stain, involves contacting a hydrolase with the food or oil stain under conditions, where the hydrolase can hydrolyze oil or fat in the stain. Reducing (M35) fat content in milk or vegetable-based dietary compositions, involves providing a composition comprising a hydrolase, milk or vegetable oil, and treating the composition with the hydrolase under conditions, where the hydrolase can hydrolyze the oil or fat in the composition, thus reducing its fat content. Catalyzing (M36) an interesterification reaction to produce new triglycerides, involves providing a composition comprising a hydrolase that can catalyze an interesterification reaction, providing a mixture of triglycerides and free fatty acids, treating the composition with the hydrolase under conditions, where the hydrolase can catalyze exchange of free fatty acids with the acyl groups of triglycerides, thus producing new triglycerides enriched in the added fatty acids. Transesterification (M37) method for preparing a margarine oil having a low trans-acid and a low intermediate chain fatty acid content, involves providing a transesterification reaction mixtu

DESCRIPTORS: recombinant hydrolase-like protein , lipase prep., plasmid, phage, phagemid, cosmid, fosmid, adeno virus, retro virus, adeno-associated virus vector, bacterial artificial chromosome, P1-derived vector, yeast artificial chromosome, mammal artificial chromosome-mediated gene transfer, expression in bacterium cell, mammal cell, fungus cell, yeast cell, insect cell, plant cell, transgenic mouse, maize, sorghum, potato, tomato, wheat, oilseed, rapeseed oil, soybean, rice, barley, grass, tobacco transgenic plant, seed, DNA probe, DNA primer, DNA array, protein array, recombinant antibody, hybridoma, computer, phenotyping, polyunsaturated fatty acid ester hydrolysis, oil, fat modification, transesterification, algorithm, appl., foodstuff, feedstuff, margarine oil prep., stereospecific, pharmaceutical, paper ind. enzyme hybridization DNA amplification cell culture culture esterification virus parvo virus arthropod animal transgenic animal mammal plant cereal grass Zea mays Solanum tuberosum fruit Lycopersicon esculentum Triticum aestivum oilseed legume Glycine max Oryza sativa Hordeum vulgare Nicotiana tabacum EC-3.1.1.3 DNA sequence (26, 03)

SECTION: BIOMANUFACTURING and BIOCATALYSIS-Biocatalyst Isolation and Characterization-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; GENETIC TECHNIQUES and APPLICATIONS-Transgenic Animals and Animal Models-BIOINFORMATICS and ANALYSIS-Software; BIOINFORMATICS and ANALYSIS-Biochips and Bioarrays-AGRICULTURAL BIOTECHNOLOGY-Plant Genetic Engineering; FOOD and FOOD-ADDITIVES-Food and Food-Additives-BIOMANUFACTURING and BIOCATALYSIS-Animal/Plant Cell Culture; BIOMANUFACTURING and BIOCATALYSIS-Biocatalyst Application-PHARMACEUTICALS-Other Pharmaceuticals

...ABSTRACT: recombinant polypeptide having (P1) and lacking a signal sequence or having heterologous signal sequence; (13) protein preparation having (P1), where the preparation is liquid/solid/gel; (14) heterodimer having (P1) and...

... time metabolic flux analysis; (37) hydrolyzing (M16) triacylglycerol (TAG), diacylglycerol (DAG) or monoacylglycerol (MAG); (38) removing /decreasing (M17) amount of TAG, DAG or MAG from composition; (39) increasing (M18) thermotolerance/thermostability...

... plant cell; (49) signal sequence having a peptide having a subsequence of (P1); (50) chimeric protein having a first domain having the signal sequence and second domain; (51) biocatalytic (M24) synthesis...

... chain fatty acid content; (67) ameliorating/preventing (M38) lipopolysaccharide- mediated toxicity; (68) detoxifying (M39) an endotoxin ; (69) deacylating (M40) 2' or 3' fatty acid chain from a lipid A; (70) hydrolyzing...

... inspection Preferred Cloning Vehicle: The cloning vehicle comprises viral vector, plasmid, phage, phagemid, cosmid, fosmid, bacteriophage or artificial chromosome. The viral vector comprises adenovirus vector, retroviral vector or adeno-associated viral vector or comprises bacterial artificial chromosome (BAC), plasmid, bacteriophage P1-derived vector (PAC), yeast artificial chromosome (YAC), or mammalian artificial chromosome (MAC). Preferred Host...

... seed. Preferred Heterodimer: The second domain is a polypeptide and the heterodimer is a fusion protein or epitope or tag. Preferred Matrix: The delivery matrix comprises a pellet. Preferred Method: Isolating...

... and contacting the polypeptide with the composition, where the polypeptide hydrolyzes triacylglycerol, diacylglycerol or monoacylglycerol. Removing (M17) or decreasing the amount of triacylglycerol, diacylglycerol or monoacylglycerol from a composition, involves providing...

... a triacylglycerol, diacylglycerol or monoacylglycerol, and contacting the polypeptide with the composition, where the polypeptide removes or decreases the amount of the triacylglycerol, diacylglycerol or monoacylglycerol. Increasing (M18) thermotolerance or thermostability ...

...hydrolase under condition, where the hydrolase can selectively hydrolyze carboxylic esters, thus treating the fabric. Removing (M34) or decreasing the amount of a food or oil stain, involves contacting a hydrolase...

DESCRIPTORS: recombinant hydrolase-like protein , lipase prep., plasmid, phage, phagemid, cosmid, fosmid, adeno virus, retro virus, adeno-associated virus vector...

...oil, soybean, rice, barley, grass, tobacco transgenic plant, seed, DNA probe, DNA primer, DNA array, protein array, recombinant antibody, hybridoma, computer, phenotyping, polyunsaturated fatty acid ester hydrolysis, oil, fat modification, transesterification...

8/9,K/7 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0385455 DBR Accession No.: 2005-31161 PATENT
NewMut isolated or recombinant polypeptide having phospholipase activity, useful for degumming oil, caustic refining of a phospholipid-containing composition, purifying a phytosterol or a triterpene, or refining a crude oil - production of a recombinant phospholipase and use of the encoding gene for preparation of a transgenic animal or a transgenic

plant and for a bioremediation application
AUTHOR: GRAMATIKOVA S; HAZLEWOOD G; LAM D; BARTON N R; STURGIS B G; ROBERTSON D E; LI J; KREPS J A; FIELDING R; BROWN R C; VASAVADA A; TAN X; BADILLO A; VAN HOEK W P; JANSSEN G; ISAAC C; BURK M J
PATENT ASSIGNEE: DIVERSA CORP 2005
PATENT NUMBER: WO 200586900 PATENT DATE: 20050922 WPI ACCESSION NO.: 2005-746919 (200576)
PRIORITY APPLIC. NO.: US 796907 APPLIC. DATE: 20040308
NATIONAL APPLIC. NO.: WO 2005US7908 APPLIC. DATE: 20050308
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated or recombinant polypeptide having phospholipase activity having comprising any one of the even-numbered amino acid sequences of SEQ ID Nos. 2-174, fully defined in the specification, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) an isolated or recombinant nucleic acid comprising a nucleic acid sequence having at least 50% sequence identity to any one of the odd-numbered nucleotide sequence of SEQ ID Nos. 1-174, fully defined in the specification, and encoding the polypeptide cited above; (2) a nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a phospholipase activity; (3) an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a phospholipase activity; (4) an isolated or recombinant phospholipase encoded by a phospholipase-encoding nucleic acid; (5) a method of amplifying a nucleic acid encoding a polypeptide having a phospholipase activity; (6) a method for making a phospholipase; (7) an expression cassette comprising the nucleic acid; (8) a vector comprising the nucleic acid; (9) a cloning vehicle comprising the nucleic acid; (10) a transformed cell comprising the nucleic acid or expression cassette; (11) a transgenic non-human animal comprising the nucleic acid; (12) a transgenic plant or seed comprising the nucleic acid; (13) an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to the nucleic acid sequence cited above or its subsequence; (14) a method of inhibiting the translation of a phospholipase message in a cell; (15) a double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of the nucleotide sequences; (16) a method of inhibiting the expression of a phospholipase in a cell; (17) a protein preparation comprising the polypeptide, where the protein preparation comprises a liquid, a solid or a gel; (18) a heterodimer comprising the polypeptide, and a second domain; (19) a homodimer comprising the polypeptide, or its subsequence; (20) an array comprising an immobilized polypeptide, or nucleic acid;(21) an isolated or recombinant antibody that specifically binds to the polypeptide; (22) a hybridoma comprising an antibody that specifically binds to the polypeptide; (23) a method of isolating or identifying a polypeptide with a phospholipase activity; (24) a method of making an anti-phospholipase antibody; (25) a method of producing a recombinant polypeptide; (26) a method for identifying a polypeptide having a phospholipase activity; (27) a method for identifying a phospholipase substrate; (28) a method of determining whether a test compound specifically binds to a polypeptide; (29) a method for identifying a modulator of a phospholipase activity; (30) a computer system comprising a processor and a data storage device stored with the polypeptide or nucleic acid sequences cited above;(31) a computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence; (32) a method for identifying a feature in a sequence; (33) a method for comparing a first sequence to a second

sequence; (34) a method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample; (35) a method of generating a variant of a nucleic acid encoding a polypeptide with a phospholipase activity; (36) a method for modifying codons in a nucleic acid encoding a polypeptide with a phospholipase activity to increase its expression in a host cell; (37) a method for producing a library of nucleic acids encoding modified phospholipase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site; (38) a method for making or modifying a small molecule; (39) a method for determining a functional fragment of a phospholipase enzyme; (40) a method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis; (41) a chimeric polypeptide comprising at least a first domain comprising signal peptide (SP), and at least a second domain comprising a heterologous polypeptide or peptide, where the heterologous polypeptide or peptide is not naturally associated with the SP; (42) an isolated or recombinant nucleic acid encoding a chimeric polypeptide; (43) a method of increasing thermotolerance or thermostability of a phospholipase polypeptide; (44) a method for overexpressing a recombinant phospholipase in a cell; (45) a method of making a transgenic plant; (46) a method of expressing a heterologous nucleic acid sequence in a plant cell; (47) a method for hydrolyzing, breaking up or disrupting a phospholipid-comprising composition; (48) a method for liquefying or removing a phospholipid-comprising composition; (49) a detergent composition comprising the polypeptide; (50) a method for washing an object; (51) a method for degumming an oil; (52) a method for converting a non-hydratable phospholipid to a hydratable form; (53) a method for caustic refining of a phospholipid-containing composition; (54) a method for purification of a phytosterol or a triterpene; (55) a method for refining a crude oil; (56) a composition having the equivalent of a phospholipase C activity comprising providing a composition comprising a polypeptide having a phospholipase activity, e.g. a phospholipase D activity, and a phosphatase enzyme; (57) a method for ameliorating or preventing lipopolysaccharide (LPS)-mediated toxicity; (58) a method for detoxifying an endotoxin ; (59) a method for deacylating a 2' or a 3 ' fatty acid chain from a lipid A; (60) a process for reducing gum mass and increasing neutral oil (triglyceride) gain through reduced oil entrapment; (61) a method for making a variant phospholipase coding sequence having increased expression in a host cell; (62) a method for making a variant phospholipase coding sequence encoding a phospholipase having increased or decreased resistance to a protease; (63) an isolated, synthetic or recombinant phospholipase encoded by the sequence made as above; (64) a method for making and expressing a protein having a biological activity whose activity is temporarily inactivated by glycosylation;(65) a method for expressing phospholipase C; (66) a cell system for expressing phospholipase C comprising a Mut+ phenotype Pichia strain comprising a heterologous phospholipase C-encoding nucleic acid operably linked to a promoter operable in the Pichia strain; and (67) a zeocin-resistant yeast cell system for expressing a heterologous protein . BIOTECHNOLOGY - Preferred Molecule: The isolated or recombinant nucleic acid comprises at least 50-100% sequence identity to those nucleotide sequences cited above. The sequence identity is over a region of at least about 10, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650,

700, 750, 800, 850, 900, 950,1000, 1050, 1100, 1150 or more residues, or the full length of a gene or a transcript. The sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default. The phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage, or of an ester linkage in a phospholipid in a vegetable oil. The vegetable oil phospholipid comprises an oilseed phospholipid. The phospholipase activity comprises a phospholipase C (PLC), phospholipase A (PLA), phospholipase B (PLB), or phospholipase D (PLD) activity. The phospholipase D activity comprises a phospholipase D1 or a phospholipase D2 activity. The phospholipase activity comprises hydrolysis of a glycoprotein . The glycoprotein comprises a potato tuber. The phospholipase activity comprises a patatin enzymatic activity, or lipid acyl hydrolase (LAH) activity. The phospholipase activity is thermostable. The polypeptide retains a phospholipase activity under conditions comprising a temperature range of 37-95, 55-85, 70-75, 70-95 or 90-85degreesC. The phospholipase activity is thermotolerant. The nucleic acid hybridizes to the above sequences at stringent conditions including a wash step comprising a wash in 0.2 X SSC at a temperature of 65degreesC for 15 minutes. The phospholipase-encoding nucleic acid is generated by amplification through polymerase chain reaction (PCR), or amplification of a gene library, specifically an environmental library. Preferred Probe: The nucleic acid probe comprises at least 10 consecutive bases of any one of the odd-numbered nucleotide sequences for SEQ ID Nos. 1-173, where the probe identifies the nucleic acid by binding or hybridization. The probe comprises an oligonucleotide comprising at least 10-50, 20-60, 30-70, 40-80, 60-100 or 50-150 consecutive bases. The sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection. The double-stranded inhibitory RNA (RNAi) molecule is 15-25 or more duplex nucleotides in length. Preferred Primer Pair: The amplification primer sequence pair is capable of amplifying the nucleic acids cited above or their subsequence. A member of the amplification primer sequence pair comprises an oligonucleotide comprising at least 10-50 consecutive bases, or about 12-25 consecutive bases of the sequence. The primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12-30 residues of the nucleotide sequences cited above, and a second member having a sequence as set forth by about the first (the 5') 12-30 or more residues of the complementary strand of the first member. Preferred Vehicle: The cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector. The cloning vehicle comprises a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC). Preferred Cell: The transformed cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell. Preferred Animal: The transgenic non-human animal is a mouse. Preferred Plant: The transgenic plant is a corn , a sorghum , a potato , a tomato , a wheat , an oilseed , a rapeseed , a soybean , a rice , a barley , a grass, a cottonseed, a palm, a sesame , a peanut , a sunflower or a tobacco plant. Preferred Polypeptide: The isolated or recombinant polypeptide has the phospholipase activity. The phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage, or catalyzing hydrolysis of an ester

linkage in a phospholipid in a vegetable oil. The vegetable oil phospholipid comprises an oilseed phospholipid. The vegetable oil phospholipid is derived from a plant oil, a high phosphorus oil, a soy oil, a canola oil, a palm oil, a cottonseed oil, a corn oil, a palm kernel-derived phospholipid, a rice bran oil, a coconut oil, a peanut oil, a sesame oil, a fish oil, an algae phospholipid, a sunflower oil, an essential oil, a fruit seed oil, a grapeseed phospholipid, an apricot phospholipid, or a borage phospholipid. The isolated or recombinant polypeptide lacks a signal sequence. The polypeptide has a heterologous signal sequence. The phospholipase activity comprises a specific activity at 37degreesC in the range from 10-100, 100-1000, 500-750, 500-1200, or 750-1000 units per milligram. The thermotolerance comprises retention of at least half of the specific activity of the phospholipase at 37degreesC after being heated to an elevated temperature. The polypeptide comprises at least one glycosylation site. The glycosylation is an N-linked glycosylation. The polypeptide is glycosylated after being expressed in P. pastoris or an S. pombe. The polypeptide retains a phospholipase activity under conditions comprising about pH 6.5, pH 6.0, pH 5.5, 5.0, pH 4.5 or 4.0. The immobilized polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube. Isolating or identifying a polypeptide with a phospholipase activity comprises providing the antibody; providing a sample comprising polypeptides; and contacting the sample with the antibody under conditions where the antibody can specifically bind to the polypeptide, thus isolating or identifying a polypeptide having a phospholipase activity. Preferred Method: Amplifying a nucleic acid encoding a polypeptide having a phospholipase activity comprises amplifying a template nucleic acid with the amplification primer sequence pair. Making a phospholipase comprises amplifying a nucleic acid with the amplification primer pair, and expressing the amplified nucleic acid. Inhibiting the translation of a phospholipase message in a cell comprises administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to the nucleotides sequence cited above. Inhibiting the expression of a phospholipase in a cell comprises administering to the cell or expressing in the cell the double-stranded inhibitory RNA (iRNA), Making an anti-phospholipase antibody comprises administering to a non-human animal the nucleic acid/polypeptide or its subsequence in an amount sufficient to generate a humoral immune response, making an anti-phospholipase antibody. Producing a recombinant polypeptide comprises providing the nucleic acid operably linked to a promoter, and expressing the nucleic acid under conditions that allow expression of the polypeptide, thus producing a recombinant polypeptide. The method further comprises transforming a host cell with the nucleic acid in the first step followed by expressing the nucleic acid. Identifying a polypeptide having a phospholipase activity comprises providing the polypeptide; providing a phospholipase substrate; and contacting the polypeptide with the substrate and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, where a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a phospholipase activity. Identifying a phospholipase substrate comprises providing the polypeptide; providing a test substrate; and contacting the polypeptide with the test substrate and detecting a decrease in the amount of substrate or an

increase in the amount of reaction product, where a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a phospholipase substrate. Determining whether a test compound specifically binds to a polypeptide comprises expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide; providing a test compound; contacting the polypeptide with the test compound; and determining whether the test compound specifically binds to the polypeptide. Identifying a modulator of a phospholipase activity comprises providing the polypeptide; providing a test compound; contacting the polypeptide with the test compound; and measuring an activity of the phospholipase, where a change in the phospholipase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the phospholipase activity. The phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product. A decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of phospholipase activity. An increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of phospholipase activity. Identifying a feature in a sequence comprises reading the sequence using a computer program, which identifies one or more features in a sequence, and identifying one or more features in the sequence with the computer program. Comparing a first sequence to a second sequence comprises reading the first sequence and the second sequence through use of a computer program which compares sequences, and determining differences between the first sequence and the second sequence with the computer program. Determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms. The method further comprises an identifier that identifies one or more features in a sequence. The method comprises reading the first sequence using a computer program and identifying one or more features in the sequence. Isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprises providing an amplification primer sequence pair; isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and combining the nucleic acid with the amplification primer pair; and amplifying nucleic acid from the environmental sample, thus isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample. The environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample. The biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell. Generating a variant of a nucleic acid encoding a polypeptide with a phospholipase activity comprises providing a template nucleic acid; and modifying, deleting or adding one or more nucleotides in the template sequence, or their combination,

to generate a variant of the template nucleic acid. The method further comprises expressing the variant nucleic acid to generate a variant phospholipase polypeptide. The modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, Gene Site Saturation Mutagenesis (GSSM), synthetic ligation reassembly (SLR) and their combination. The modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis , artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and their combination. The method is iteratively repeated until a phospholipase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced. The variant phospholipase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature. The variant phospholipase polypeptide has increased glycosylation as compared to the phospholipase encoded by a template nucleic acid. The variant phospholipase polypeptide has a phospholipase activity under a high temperature, where the phospholipase encoded by the template nucleic acid is not active under the high temperature. The method is iteratively repeated until a phospholipase coding sequence having an altered codon usage from that of the template nucleic acid is produced. The method is iteratively repeated until a phospholipase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced. Modifying codons in a nucleic acid encoding a polypeptide with a phospholipase activity to increase its expression in a host cell comprises providing a nucleic acid encoding a polypeptide with a phospholipase activity; identifying a non-preferred or a less preferred codon in the nucleic acid; and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, where a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thus modifying the nucleic acid to increase its expression in a host cell. Modifying codons in a nucleic acid encoding a phospholipase polypeptide comprises providing a nucleic acid encoding a polypeptide with a phospholipase activity; and identifying a codon in the nucleic acid and replacing it with a different codon encoding the same amino acid as the replaced codon. Modifying codons in a nucleic acid encoding a phospholipase polypeptide to increase or decrease its expression in a host cell comprises providing a nucleic acid encoding a phospholipase polypeptide; and identifying a non-preferred or a less preferred codon in the nucleic acid and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, where a preferred codon is a codon over-represented or under-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell. Producing a library of nucleic acids encoding modified phospholipase

active sites or substrate binding sites comprises providing a first nucleic acid encoding a first active site or first substrate binding site, providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at targeted codons in the first nucleic acid; and using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized. The method comprises mutagenizing the first nucleic acid or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis , artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and their combination. Making a small molecule comprises providing biosynthetic enzymes capable of synthesizing or modifying a small molecule; providing a substrate for at least one of the enzymes; and reacting the substrate with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reaction Modifying a small molecule comprises providing a phospholipase enzyme; providing a small molecule; and reacting the enzyme with the small molecule under conditions that facilitate an enzymatic reaction catalyzed by the phospholipase enzyme. The method further comprises testing the library to determine if a particular modified small molecule, which exhibits a desired activity is present within the library. Determining a functional fragment of a phospholipase enzyme comprises providing a phospholipase enzyme; and deleting amino acid residues from the sequence and testing the remaining subsequence for a phospholipase activity. The method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis comprises making a modified cell by modifying the genetic composition of a cell, where the genetic composition is modified by addition to the cell of a nucleic acid; culturing the modified cell to generate a plurality of modified cells; measuring at least one metabolic parameter of the cell by monitoring the cell culture in real time; and analyzing the data to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions. The genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene. The method further comprises selecting a cell comprising a newly engineered phenotype. The method further comprises culturing the selected cell. Increasing thermotolerance or thermostability of a phospholipase polypeptide comprises glycosylating a phospholipase. Overexpressing a recombinant phospholipase in a cell comprises expressing a vector comprising a nucleic acid sequence, where overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector. Making a transgenic plant comprises introducing a heterologous nucleic acid sequence into the cell, where the heterologous nucleic sequence, thus producing a transformed plant cell; producing a transgenic plant from the transformed cell. The method further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts. The method comprises introducing the heterologous nucleic acid sequence directly

to plant tissue by DNA particle bombardment or by using an Agrobacterium tumefaciens host. Expressing a heterologous nucleic acid sequence in a plant cell comprises transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter; and growing the plant under conditions where the heterologous nucleic acids sequence is expressed in the plant cell. Hydrolyzing, breaking up or disrupting a phospholipid-comprising composition comprises providing a polypeptide having a phospholipase activity; providing a composition comprising a phospholipid; and contacting the polypeptide with the composition under conditions where the phospholipase hydrolyzes, breaks up or disrupts the phospholipid-comprising composition. Liquefying or removing a phospholipid-comprising composition comprises providing a polypeptide having a phospholipase activity; providing a composition comprising a phospholipid; and contacting the polypeptide with the composition under conditions where the phospholipase removes or liquefies the phospholipid-comprising composition. Washing an object comprises providing a composition comprising a polypeptide having a phospholipase activity; providing an object; and contacting the polypeptide and the object under conditions where the composition can wash the object. Degumming an oil comprises providing a composition comprising a polypeptide having a phospholipase activity; providing a composition comprising a phospholipid-containing fat or oil; and contacting the polypeptide and the composition under conditions where the polypeptide can catalyze the hydrolysis of a phospholipid in the composition. The oil-comprising composition comprises a plant, an animal, algae or a fish oil or fat. The plant oil comprises rice bran oil, soybean oil, a rapeseed oil, a corn oil, oil from a palm kernel, canola oil, sunflower oil, sesame oil or a peanut oil. The polypeptide hyd

DESCRIPTORS: recombinant phospholipase prep., isol., immobilization, bacterium artificial chromosome, plasmid, phage, yeast artificial chromosome, mammal artificial chromosome vector-mediated gene transfer, expression in host cell, computer bioinformatic hardware, computer bioinformatic software, maize, sorghum, potato, tomato, wheat, oilseed, rape, soybean, rice, barley, grass, palm, sesame, peanut, sunflower, tobacco, transgenic plant prep., particle bombardment, Agrobacterium tumefaciens vector-mediated gene expression, antisense oligonucleotide, transgenic mouse prep., DNA probe, monoclonal antibody prep., hybridoma, appl., oil degradation, bioremediation enzyme bioinformatics transgenic animal plant cereal grass Zea mays Solanum tuberosum fruit Lycopersicon esculentum Triticum aestivum oilseed Brassica napus legume Glycine max Oryza sativa Hordeum vulgare Sesamum indicum Arachis hypogaea Helianthus annuus Nicotiana tabacum bacterium hybridization cell culture pollutant degradation (24, 51)

SECTION: BIOMANUFACTURING and BIOCATALYSIS-Biocatalyst Application-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; GENETIC TECHNIQUES and APPLICATIONS-Transgenic Animals and Animal Models-BIOINFORMATICS and ANALYSIS-Hardware; BIOINFORMATICS and ANALYSIS-Software-BIOMANUFACTURING and BIOCATALYSIS-Animal/Plant Cell Culture; BIOMANUFACTURING and BIOCATALYSIS-Biocatalyst Isolation and Characterization-WASTE-DISPOSAL AND BIOREMEDIATION-Environmental Biotechnology; PHARMACEUTICALS-Antibodies-AGRICULTURAL BIOTECHNOLOGY-Plant Genetic Engineering

...ABSTRACT: 16) a method of inhibiting the expression of a phospholipase in a cell; (17) a protein preparation comprising the polypeptide, where the protein preparation comprises a liquid, a solid or a gel;

(18) a heterodimer comprising the polypeptide...

... hydrolyzing, breaking up or disrupting a phospholipid-comprising composition; (48) a method for liquefying or removing a phospholipid-comprising composition; (49) a detergent composition comprising the polypeptide; (50) a method for...

... method for ameliorating or preventing lipopolysaccharide (LPS)-mediated toxicity; (58) a method for detoxifying an endotoxin ; (59) a method for deacylating a 2' or a 3 ' fatty acid chain from a...

... encoded by the sequence made as above; (64) a method for making and expressing a protein having a biological activity whose activity is temporarily inactivated by glycosylation;(65) a method for...

... the Pichia strain; and (67) a zeocin-resistant yeast cell system for expressing a heterologous protein . BIOTECHNOLOGY - Preferred Molecule: The isolated or recombinant nucleic acid comprises at least 50-100% sequence...

... a phospholipase D1 or a phospholipase D2 activity. The phospholipase activity comprises hydrolysis of a glycoprotein . The glycoprotein comprises a potato tuber. The phospholipase activity comprises a patatin enzymatic activity, or lipid acyl...

... comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector comprises an adenovirus vector, a retroviral vector or...

... associated viral vector. The cloning vehicle comprises a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC...

...conditions where the phospholipase hydrolyzes, breaks up or disrupts the phospholipid-comprising composition. Liquefying or removing a phospholipid-comprising composition comprises providing a polypeptide having a phospholipase activity; providing a composition comprising a phospholipid; and contacting the polypeptide with the composition under conditions where the phospholipase removes or liquefies the phospholipid-comprising composition. Washing an object comprises providing a composition comprising a...

8/9,K/8 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0374960 DBR Accession No.: 2005-20666 PATENT
Assuring identity of a regulated bacteriophage preparation, for industrial and/or therapeutic applications, by comparing nuclease and proteases digests in phage plaques for disparity in relative molecular weights - production and characterization of a phage composition useful for a gene therapy application
AUTHOR: SULAKVELIDZE A; SOZHAMANNAN S; VOELKER L
PATENT ASSIGNEE: INTRALYTIX INC 2005
PATENT NUMBER: WO 200559161 PATENT DATE: 20050630 WPI ACCESSION NO.:

2005-467128 (200547)
PRIORITY APPLIC. NO.: US 529772 APPLIC. DATE: 20031217
NATIONAL APPLIC. NO.: WO 2004US42475 APPLIC. DATE: 20041217
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Assuring identity of a regulated bacteriophage preparation comprises obtaining at least two phage plaques from the preparation; and comparing nuclease and proteases digests of the plaques with each other and with that of a reference strain; where disparity in relative molecular weight indicates lack of identity. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a method of preparing a lytic bacteriophage preparation for therapeutic use; and (2) a phage fully given in the specification. BIOTECHNOLOGY - Preferred Method: The method further comprises determining the presence of bacteria or toxin in the preparation, or testing at least one of total protein , total nitrogen, pH, specific gravity, total dissolved solids, percent ash and total non-volatile solids of the preparation. Preparing a lytic bacteriophage preparation for therapeutic use comprises growing a population of bacteriophage in a culture of host bacteria; removing host bacteria from the population of bacteriophage ; producing a monoclonal population of the bacteriophage ; testing the population of bacteriophage for bacterial contamination; testing the population of bacteriophage for endotoxin contamination; and testing the population of bacteriophage for contamination by lysogenic phage. USE - The methods are useful for selecting and monitoring and controlling the safety and efficacy of successive batches of bacteriophage prepared for industrial and/or therapeutic applications.(32 pages)
DESCRIPTORS: phage comp. prep., characterization, appl., gene therapy (24, 33)
SECTION: THERAPEUTICS-Gene Therapy-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis

Assuring identity of a regulated bacteriophage preparation, for industrial and/or therapeutic applications, by comparing nuclease and proteases digests in phage...
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Assuring identity of a regulated bacteriophage preparation comprises obtaining at least two phage plaques from the preparation; and comparing nuclease and...

... INDEPENDENT CLAIMS are also included for the following: (1) a method of preparing a lytic bacteriophage preparation for therapeutic use; and (2) a phage fully given in the specification. BIOTECHNOLOGY - Preferred ...

... presence of bacteria or toxin in the preparation, or testing at least one of total protein , total nitrogen, pH, specific gravity, total dissolved solids, percent ash and total non-volatile solids of the preparation. Preparing a lytic bacteriophage preparation for therapeutic use comprises growing a population of bacteriophage in a culture of host bacteria; removing host bacteria from the population of bacteriophage ; producing a monoclonal population of the bacteriophage ; testing the population of bacteriophage for bacterial contamination; testing the population of bacteriophage for endotoxin contamination; and testing the population of bacteriophage for contamination by lysogenic phage. USE - The methods are useful for selecting and monitoring and controlling the safety and efficacy of successive batches of bacteriophage prepared for industrial and/or

therapeutic applications.(32 pages)

8/9,K/9 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0333474 DBR Accession No.: 2004-05766 PATENT
Method for detecting and removing endotoxins, useful for treating e.g. recombinantly produced pharmaceuticals or nucleic acid, by incubation with phage tail protein - involving vector-mediated gene transfer and expression in host cell for use in gene therapy
AUTHOR: SCHUETZ M; MEYER R; GRALLERT H; MILLER S
PATENT ASSIGNEE: PROFOS AG 2003
PATENT NUMBER: WO 200401418 PATENT DATE: 20031231 WPI ACCESSION NO.: 2004-071780 (200407)
PRIORITY APPLIC. NO.: DE 1007793 APPLIC. DATE: 20030224
NATIONAL APPLIC. NO.: WO 2003DE2096 APPLIC. DATE: 20030624
LANGUAGE: German
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Method for detecting endotoxins (I) comprises incubating a sample with a bacteriophage tail protein (II), then detecting any (I) bound to (II). DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method of removing (I) from a sample by incubation or contact with (II) that is immobilized, non-specifically or in a targeted manner, on a solid carrier. WIDER DISCLOSURE - This describes (1) phage proteins coupled at either end, especially the C-terminus, to a tag, particularly one that has a surface-exposed Cys residue for targeted biotinylation, and (2) nucleic acid encoding the proteins of (1). USE - The method is used to detect endotoxin (I) in e.g. recombinantly produced pharmaceuticals, gene therapy agents or materials intended for injection, also in research materials (nucleic acids) used in transfection experiments. When (II) is immobilized on a solid carrier, then method can be used for removing (I). ADVANTAGE - The method is applicable to all aqueous solutions; is superior to known detection and purification methods, and avoids the difficult preparation of antibodies directed against the core oligosaccharide of lipopolysaccharide. EXAMPLE - The p12 phage tail protein was reacted with sulfo-N-hydroxysuccinimide-LC-LC-biotin , then incubated with streptavidin-loaded chromatography material. A solution (1 ml) of bovine serum albumen (BSA) containing 10 EU/ml of endotoxin was stirred for 1 hour at room temperature with the p12-containing material (50 microlitres), then centrifuged and endotoxin concentration in the supernatant measured. Removal of endotoxin was 86% and recovery of albumen 90%.(41 pages)
DESCRIPTORS: recombinant phage tail protein prep., isol., vector-mediated gene transfer, expression in host cell, appl. gene therapy, pharmaceutical ind. (23, 11)
SECTION: THERAPEUTICS- Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; PHARMACEUTICALS-Other Pharmaceuticals-THERAPEUTICS-Gene Therapy
Method for detecting and removing endotoxins, useful for treating e.g. recombinantly produced pharmaceuticals or nucleic acid, by incubation with phage tail protein - involving vector-mediated gene transfer and expression in host cell for use in gene therapy
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Method for detecting endotoxins (I) comprises incubating a sample with a bacteriophage tail protein (II), then detecting any (I) bound to (II). DETAILED DESCRIPTION - An

INDEPENDENT CLAIM is also included for a method of removing (I) from a sample by incubation or contact with (II) that is immobilized, non-specifically...

... 2) nucleic acid encoding the proteins of (1). USE - The method is used to detect endotoxin (I) in e.g. recombinantly produced pharmaceuticals, gene therapy agents or materials intended for injection...

... experiments. When (II) is immobilized on a solid carrier, then method can be used for removing (I). ADVANTAGE - The method is applicable to all aqueous solutions; is superior to known detection...

... preparation of antibodies directed against the core oligosaccharide of lipopolysaccharide. EXAMPLE - The p12 phage tail protein was reacted with sulfo-N-hydroxysuccinimide-LC-LC-biotin, then incubated with streptavidin-loaded chromatography material. A solution (1 ml) of bovine serum albumen (BSA) containing 10 EU/ml of endotoxin was stirred for 1 hour at room temperature with the p12-containing material (50 microlitres), then centrifuged and endotoxin concentration in the supernatant measured. Removal of endotoxin was 86% and recovery of albumen 90%.(41 pages)
DESCRIPTORS: recombinant phage tail protein prep., isol., vector-mediated gene transfer, expression in host cell, appl. gene therapy, pharmaceutical ind...
SECTION: THERAPEUTICS- Protein Therapeutics...
? t s9/9,k/1-5

9/9,K/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06035633 BIOSIS NO.: 198070067120
BACTERIO PHAGE T-4D RECEPTOR AND THE ESCHERICHIA-COLI CELL WALL STRUCTURE BINDING OF ENDO TOXIN-LIKE PARTICLES TO THE CELL WALL
AUTHOR: ZORZOPULOS J (Reprint); DELONG S; CHAPMAN V; KOZLOFF L M
AUTHOR ADDRESS: DEP MICROBIOL IMMUNOL, UNIV COLO HEALTH SCI CENT, DENVER, COLO 80262, USA**USA
JOURNAL: Journal of Bacteriology 142 (3): p982-991 1980
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A variety of degradative treatments were used to investigate the nature of the structure and components of the cell walls of E. coli B. The binding and localization of the endotoxin -like particles found on the cell walls were of special interest because some of them are associated with the site where the inner tail tube of bacteriophage T4D penetrates the cell wall. Modified cell walls were obtained by heating a suspension of bacterial cells originally in 0.1 M phosphate, pH 7.0, after the addition of 12.5 M NaOH to a final concentration of 0.25 M. With regard to the endotoxin -like particles, it was found that: at least part of them still remained bound to the modified cell wall after the alkali treatment; the subsequent incubation of alkali-treated cell walls with lysozyme destroyed the bacterial form and released a complex of endotoxin -like particles together with a fibrous material; treatment

with 45% phenol at 70.degree. C removed the endotoxin -like particles from the surface of the alkali-treated cell walls, but most of the fibrous material was left on the cell wall; and incubation of alkali-treated cell walls with 5 mM EDTA at 20.degree. C also removed the endotoxin -like particles, but did not disrupt the rodlike bacterial form. If the EDTA treatment was performed at 55.degree. C, the bacterium-like form was destroyed. These differential sensitivities to EDTA suggested that loosely bound divalent metal ions normally hold these endotoxin -like particles on the cell wall surface, but that probably more tightly bound metal ions are involved in the determination of cell shape. Analysis of the protein components of the alkali-treated cell walls showed that only 1 protein was present in significant amounts, and this protein had an electrophoretic mobility similar to that of the Braun lipoprotein. This protein was released from the alkali-treated cell walls upon heating with 2% sodium dodecyl sulfate at 100.degree. C. Phospholipids were also absent from this structure. The distribution of the remaining cell wall components on the alkali-treated cell walls is discussed.

REGISTRY NUMBERS: 9001-63-2: LYSOZYME
DESCRIPTORS: BRAUNS LIPO PROTEIN PROTEIN DIVALENT METAL ION LYSOZYME
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Microbiology; Physiology; Toxicology
BIOSYSTEMATIC NAMES: Viruses--Microorganisms; Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
COMMON TAXONOMIC TERMS: Viruses; Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: LYSOZYME
CONCEPT CODES:
10010 Comparative biochemistry
10054 Biochemistry methods - Proteins, peptides and amino acids
10056 Biochemistry methods - Lipids
10058 Biochemistry methods - Carbohydrates
10060 Biochemistry studies - General
10064 Biochemistry studies - Proteins, peptides and amino acids
10066 Biochemistry studies - Lipids
10068 Biochemistry studies - Carbohydrates
10069 Biochemistry studies - Minerals
10504 Biophysics - Methods and techniques
10614 External effects - Temperature as a primary variable
10618 External effects - Temperature as a primary variable - hot
10804 Enzymes - Methods
12100 Movement
22501 Toxicology - General and methods
23001 Temperature - General measurement and methods
31000 Physiology and biochemistry of bacteria
32000 Microbiological apparatus, methods and media
33504 Virology - Bacteriophage
BIOSYSTEMATIC CODES:
03000 Viruses
06702 Enterobacteriaceae

...ABSTRACT: components of the cell walls of E. coli B. The binding and localization of the endotoxin -like particles found on the cell walls were of special interest because some of them are associated with the site where the inner tail tube of bacteriophage T4D penetrates the cell

wall. Modified cell walls were obtained by heating a suspension of...

...5 M NaOH to a final concentration of 0.25 M. With regard to the endotoxin -like particles, it was found that: at least part of them still remained bound to the modified cell wall after the alkali treatment; the subsequent incubation of alkali-treated cell walls with lysozyme destroyed the bacterial form and released a complex of endotoxin -like particles together with a fibrous material; treatment with 45% phenol at 70.degree. C removed the endotoxin -like particles from the surface of the alkali-treated cell walls, but most of the fibrous material was left on the cell wall; and incubation of alkali-treated cell walls with 5 mM EDTA at 20.degree. C also removed the endotoxin -like particles, but did not disrupt the rodlike bacterial form. If the EDTA treatment was...

...These differential sensitivities to EDTA suggested that loosely bound divalent metal ions normally hold these endotoxin -like particles on the cell wall surface, but that probably more tightly bound metal ions are involved in the determination of cell shape. Analysis of the protein components of the alkali-treated cell walls showed that only 1 protein was present in significant amounts, and this protein had an electrophoretic mobility similar to that of the Braun lipoprotein. This protein was released from the alkali-treated cell walls upon heating with 2% sodium dodecyl sulfate...

DESCRIPTORS: BRAUNS LIPO PROTEIN PROTEIN DIVALENT METAL ION LYSOZYME

02019394 EMBASE No: 1981006563

Bacteriophage T4D receptor and the Escherichia coli cell wall structure: binding of endotoxin -like particles to the cell wall

Zorzopulos J.; DeLong S.; Chapman V.; Kozloff L.M.
Dept. Microbiol. Immunol., Univ. Colorado Hlth Sci. Cent., Denver, Colo. 80262 United States

Journal of Bacteriology (J. BACTERIOL.) (United States) 1980, 142/3 (982-991)
CODEN: JOBAA
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH

A variety of degradative treatments have been used to investigate the nature of the structure and components of the cell walls of Escherichia coli B. The binding and localization of the endotoxin -like particles found on the cell walls were of special interest because some of them are associated with the site where the inner tail tube of bacteriophage T4D penetrates the cell wall. Modified cell walls were obtained by heating a suspension of bacterial cells originally in 0.1 M phosphate, pH 7.0, after the addition of 12.5 M NaOH to a final concentration of 0.25 M. With regard to the endotoxin -like particles, it was found that: at least part of them still remained bound to the modified cell wall after the alkali treatment; the subsequent incubation of alkali-treated cell walls with lysozyme destroyed the bacterial form and released a complex of endotoxin -like particles together with a fibrous material; on the other hand, treatment with 45% phenol at 70degreeC removed the endotoxin -like particles from the surface of the alkali-treated cell walls, but most of the fibrous

material was left on the cell wall; and (iv) incubation of alkali-treated cell walls with 5 mM ethylenediaminetetraacetic acid at 20degreeC also removed the endotoxin -like particles, but did not disrupt the rodlike bacterial form. However, if the ethylenediaminetetraacetic acid treatment was performed at 55degreeC, the bacterium-like form was destroyed. These differential sensitivities to ethylenediaminetetraacetic acid suggested that loosely bound divalent metal ions normally hold these endotoxin -like particles on the cell wall surface, but that probably more tightly bound metal ions are involved in the determination of cell shape. Analysis of the protein components of the alkali-treated cell walls showed that only one protein was present in significant amounts, and this protein had an electrophoretic mobility similar to that of the Braun lipoprotein. This protein was released from the alkali-treated cell walls upon heating with 2% sodium dodecyl sulfate at 100degreeC. Phospholipids were also absent from this structure. The distribution of the remaining cell wall components on the alkali-treated cell walls is discussed.

DRUG DESCRIPTORS:
* bacteriophage receptor
MEDICAL DESCRIPTORS:
* bacteriophage ; *bacterial cell wall; *escherichia coli
virus cell interaction; in vitro study; animal experiment
SECTION HEADINGS:
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
047 Virology

Bacteriophage T4D receptor and the Escherichia coli cell wall structure: binding of endotoxin -like particles to the cell wall

...components of the cell walls of Escherichia coli B. The binding and localization of the endotoxin -like particles found on the cell walls were of special interest because some of them are associated with the site where the inner tail tube of bacteriophage T4D penetrates the cell wall. Modified cell walls were obtained by heating a suspension of...

...5 M NaOH to a final concentration of 0.25 M. With regard to the endotoxin -like particles, it was found that: at least part of them still remained bound to the modified cell wall after the alkali treatment; the subsequent incubation of alkali-treated cell walls with lysozyme destroyed the bacterial form and released a complex of endotoxin -like particles together with a fibrous material; on the other hand, treatment with 45% phenol at 70degreeC removed the endotoxin -like particles from the surface of the alkali-treated cell walls, but most of the fibrous material was left on the cell wall; and (iv) incubation of alkali-treated cell walls with 5 mM ethylenediaminetetraacetic acid at 20degreeC also removed the endotoxin -like particles, but did not disrupt the rodlike bacterial form. However, if the ethylenediaminetetraacetic acid...

...differential sensitivities to ethylenediaminetetraacetic acid suggested that loosely bound divalent metal ions normally hold these endotoxin -like particles on the cell wall surface, but that probably more tightly bound metal ions are involved in the determination of cell shape. Analysis of the protein components of the alkali-treated cell walls showed that only one protein was present in significant amounts, and this protein had an electrophoretic mobility similar to that of the Braun lipoprotein. This protein was released from the alkali-treated cell walls upon heating with 2% sodium dodecyl sulfate...

DRUG DESCRIPTORS:
* bacteriophage receptor
MEDICAL DESCRIPTORS:
* bacteriophage ; *bacterial cell wall; *escherichia coli

9/9,K/3 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0376837 DBR Accession No.: 2005-22543 PATENT
For the detection of endotoxins, in a sample, the sample is incubated with bacteriophage tail proteins where the endotoxins bond to the tail proteins for detection - phage tail protein sample incubation for endotoxin detection and gene therapy
AUTHOR: MEYER R; SCHUETZ M; GRALLERT H; GRASSL R; MILLER S
PATENT ASSIGNEE: PROFOS AG 2005
PATENT NUMBER: WO 200562051 PATENT DATE: 20050707 WPI ACCESSION NO.: 2005-522260 (200553)
PRIORITY APPLIC. NO.: DE 1060844 APPLIC. DATE: 20031220
NATIONAL APPLIC. NO.: WO 2004DE2778 APPLIC. DATE: 20041220
LANGUAGE: German
ABSTRACT: DERWENT ABSTRACT: NOVELTY - For the detection of endotoxins in a sample, the sample is incubated with bacteriophage tail proteins. The endotoxin , bonded to the tail protein , is detected by enzyme linked immunoabsorbant assay (ELISA), chemical or enzyme detection reactions or split endotoxin components or capacity measurement. USE - The technique is for the detection of endotoxins which could have an adverse effect on genetically produced pharmaceuticals, genetic therapeutics, or substances injected into humans and animals. Endotoxins can also affect research materials in transfection experiments with mammalian cells. ADVANTAGE - The technique gives a simple and standardized method for the detection of endotoxins in solutions and samples.(63 pages)
DESCRIPTORS: phage tail protein sample incubation , ELISA, chemical, enzyme detection reaction, split endotoxin component, capacity measurement, appl. endotoxin det., human, animal gene therapy, genetically produced pharmaceutical, injected substance adverse effecte evaluation, transfection experiment analysis immunoassay toxin protein mammal (24, 36)
SECTION: THERAPEUTICS-Gene Therapy-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis

For the detection of endotoxins, in a sample, the sample is incubated with bacteriophage tail proteins where the endotoxins bond to the tail proteins for detection - phage tail protein sample incubation for endotoxin detection and gene therapy
ABSTRACT: DERWENT ABSTRACT: NOVELTY - For the detection of endotoxins in a sample, the sample is incubated with bacteriophage tail proteins. The endotoxin , bonded to the tail protein , is detected by enzyme linked immunoabsorbant assay (ELISA), chemical or enzyme detection reactions or split endotoxin components or capacity measurement. USE - The technique is for the detection of endotoxins which could...
DESCRIPTORS: phage tail protein sample incubation , ELISA, chemical, enzyme detection reaction, split endotoxin component, capacity measurement, appl. endotoxin det., human, animal gene therapy, genetically produced pharmaceutical, injected substance adverse effecte

evaluation, transfection experiment analysis immunoassay toxin protein mammal (24, 36)

9/9,K/4 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0333474 DBR Accession No.: 2004-05766 PATENT
Method for detecting and removing endotoxins, useful for treating e.g. recombinantly produced pharmaceuticals or nucleic acid, by incubation with phage tail protein - involving vector-mediated gene transfer and expression in host cell for use in gene therapy
AUTHOR: SCHUETZ M; MEYER R; GRALLERT H; MILLER S
PATENT ASSIGNEE: PROFOS AG 2003
PATENT NUMBER: WO 200401418 PATENT DATE: 20031231 WPI ACCESSION NO.: 2004-071780 (200407)
PRIORITY APPLIC. NO.: DE 1007793 APPLIC. DATE: 20030224
NATIONAL APPLIC. NO.: WO 2003DE2096 APPLIC. DATE: 20030624
LANGUAGE: German
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Method for detecting endotoxins (I) comprises incubating a sample with a bacteriophage tail protein (II), then detecting any (I) bound to (II). DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method of removing (I) from a sample by incubation or contact with (II) that is immobilized, non-specifically or in a targeted manner, on a solid carrier. WIDER DISCLOSURE - This describes (1) phage proteins coupled at either end, especially the C-terminus, to a tag, particularly one that has a surface-exposed Cys residue for targeted biotinylation, and (2) nucleic acid encoding the proteins of (1). USE - The method is used to detect endotoxin (I) in e.g. recombinantly produced pharmaceuticals, gene therapy agents or materials intended for injection, also in research materials (nucleic acids) used in transfection experiments. When (II) is immobilized on a solid carrier, then method can be used for removing (I). ADVANTAGE - The method is applicable to all aqueous solutions; is superior to known detection and purification methods, and avoids the difficult preparation of antibodies directed against the core oligosaccharide of lipopolysaccharide. EXAMPLE - The p12 phage tail protein was reacted with sulfo-N-hydroxysuccinimide-LC-LC-biotin, then incubated with streptavidin-loaded chromatography material. A solution (1 ml) of bovine serum albumen (BSA) containing 10 EU/ml of endotoxin was stirred for 1 hour at room temperature with the p12-containing material (50 microlitres), then centrifuged and endotoxin concentration in the supernatant measured. Removal of endotoxin was 86% and recovery of albumen 90%.(41 pages)
DESCRIPTORS: recombinant phage tail protein prep., isol., vector-mediated gene transfer, expression in host cell, appl. gene therapy, pharmaceutical ind. (23, 11)
SECTION: THERAPEUTICS- Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; PHARMACEUTICALS-Other Pharmaceuticals-THERAPEUTICS-Gene Therapy
...and removing endotoxins, useful for treating e.g. recombinantly produced pharmaceuticals or nucleic acid, by incubation with phage tail protein - involving vector-mediated gene transfer and expression in host cell for use in gene therapy
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Method for detecting endotoxins (I) comprises incubating a sample with a bacteriophage tail protein

(II), then detecting any (I) bound to (II). DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method of removing (I) from a sample by incubation or contact with (II) that is immobilized, non-specifically or in a targeted manner, on...

... 2) nucleic acid encoding the proteins of (1). USE - The method is used to detect endotoxin (I) in e.g. recombinantly produced pharmaceuticals, gene therapy agents or materials intended for injection...

... preparation of antibodies directed against the core oligosaccharide of lipopolysaccharide. EXAMPLE - The p12 phage tail protein was reacted with sulfo-N-hydroxysuccinimide-LC-LC-biotin, then incubated with streptavidin-loaded chromatography material. A solution (1 ml) of bovine serum albumen (BSA) containing 10 EU/ml of endotoxin was stirred for 1 hour at room temperature with the p12-containing material (50 microlitres), then centrifuged and endotoxin concentration in the supernatant measured. Removal of endotoxin was 86% and recovery of albumen 90%.(41 pages)
DESCRIPTORS: recombinant phage tail protein prep., isol., vector-mediated gene transfer, expression in host cell, appl. gene therapy, pharmaceutical ind...
SECTION: THERAPEUTICS- Protein Therapeutics...

9/9,K/5 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0308946 DBR Accession No.: 2003-10731 PATENT
Selective enrichment of bacteria or their components, useful e.g. for isolating nucleic acid, by binding to bacteriophage , or their proteins, immobilized on a carrier - recombinant vector plasmid pET21a-mediated gene transfer and expression in Escherichia coli for use in polysaccharide isolation
AUTHOR: KARETH M S; GRASSL R; MEYER R; FRICK S; ROBL I; ZANDER T; MILLER S
PATENT ASSIGNEE: PROFOS AG 2003
PATENT NUMBER: WO 2003000888 PATENT DATE: 20030103 WPI ACCESSION NO.: 2003-229338 (200322)
PRIORITY APPLIC. NO.: DE 1029815 APPLIC. DATE: 20010624
NATIONAL APPLIC. NO.: WO 2002DE2302 APPLIC. DATE: 20020624
LANGUAGE: German
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Method for selective enrichment of bacteria (A), or their components, comprises contacting the sample with bacteriophages and/or their proteins (B); incubating the mixture with a solid carrier having on its surface at least one coupling group for (B); and separating the carrier having (A), bound to (B), attached to it. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a method in which the (A)-containing sample is contacted with magnetic particles having (B) attached to the surface and separation of the particles; (2) Magnetic particles coated with (B); (3) Phage proteins (PP) that include a Strep- or His-tag; (4) Nucleic acid encoding PP; (5) Three peptides of 19, 19 and 539 amino acids (reproduced), designated sequences (S6), (S7) and (S8); and (6) Kit for enriching (A) comprising the particles of (2) and/or the PP of (3). BIOTECHNOLOGY - PP are prepared by recombinant DNA methods. The

specification includes primer sequences for amplification of p12 with an attached Strep-tag. The amplicon is then cloned into pET21a and the recombinant plasmid expressed in Escherichia coli BL21(DE3) to produce tagged p12. USE - The method is particularly used to isolate nucleic acid (particularly for plasmid preparation), lipopolysaccharides, endotoxins and exopolysaccharides. ADVANTAGE - (A) can be enriched selectively (depending on which (B) are used) in a fully automated process that can be incorporated into existing analytical/isolation procedures. EXAMPLE - A recombinant T4 phage p12 protein that included a N-Strep-tag was incubated for 1 hour with a culture of Escherichia coli. The cell- protein mixture was then incubated with magnetic beads coated with streptavidin for 90 minutes. The beads were separated magnetically and their beta-galactosidase activity measured. This was about 150 (units not specified), compared with zero when beads without the p12 protein were used (background) and about 50 for beads that had been coated with the p12 protein first and then incubated with the bacterial suspension. (40 pages)

DESCRIPTORS: recombinant vector plasmid pET21a-mediated gene transfer, expression in Escherichia coli, DNA primer, appl. lipopolysaccharide, endotoxin isol. bacterium DNA amplification lipid polysaccharide toxin protein DNA sequence (22, 18)

SECTION: OTHER CHEMICALS-Polymers-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis

...of bacteria or their components, useful e.g. for isolating nucleic acid, by binding to bacteriophage , or their proteins, immobilized on a carrier - recombinant vector plasmid pET21a-mediated gene transfer and ...

...ABSTRACT: A), or their components, comprises contacting the sample with bacteriophages and/or their proteins (B); incubating the mixture with a solid carrier having on its surface at least one coupling group...

... that can be incorporated into existing analytical/isolation procedures. EXAMPLE - A recombinant T4 phage p12 protein that included a N-Strep-tag was incubated for 1 hour with a culture of Escherichia coli. The cell- protein mixture was then incubated with magnetic beads coated with streptavidin for 90 minutes. The beads were separated magnetically and...

... This was about 150 (units not specified), compared with zero when beads without the p12 protein were used (background) and about 50 for beads that had been coated with the p12 protein first and then incubated with the bacterial suspension. (40 pages)

DESCRIPTORS: recombinant vector plasmid pET21a-mediated gene transfer, expression in Escherichia coli, DNA primer, appl. lipopolysaccharide, endotoxin isol. bacterium DNA amplification lipid polysaccharide toxin protein DNA sequence (22, 18)

```
? s lipopolysaccharide
     S10  268205  LIPOPOLYSACCHARIDE
? s s10 and s2
          268205  S10
            1724  S2
     S11      19  S10 AND S2
? s s10 and s4
          268205  S10
           56941  S4
     S12     543  S10 AND S4
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? s s11 and detect?
             19  S11
        6789758  DETECT?
     S13       2  S11 AND DETECT?
? s s11 and incubat?
             19  S11
        1228212  INCUBAT?
     S14       1  S11 AND INCUBAT?
? s s11 and incubat?
             19  S11
        1228212  INCUBAT?
     S15       1  S11 AND INCUBAT?
? s s12 and detect?
            543  S12
        6789758  DETECT?
     S16      73  S12 AND DETECT?
? s s12 and remov?
            543  S12
        2029770  REMOV?
     S17      27  S12 AND REMOV?
? s s12 and salmonella
            543  S12
         335824  SALMONELLA
     S18     137  S12 AND SALMONELLA
? s s12 and incubat?
            543  S12
        1228212  INCUBAT?
     S19      35  S12 AND INCUBAT?
? t s11/9,k/1-10

 11/9,K/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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10818044   BIOSIS NO.: 199192063815
AMINO ACID SEQUENCE OF THE BACTERIOPHAGE T5 GENE A2 PROTEIN
AUTHOR: SNYDER C E JR (Reprint)
AUTHOR ADDRESS: PEPTIDE SYNTHESIS LAB, CLINICAL IMMUNOL SERVICES,
  PRI/DYNCORP, NCI-FCR, FREDERICK, MD 21702, USA**USA
JOURNAL: Biochemical and Biophysical Research Communications  177 (3): p
1240-1246 1991
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The complete amino acid sequence of the bacteriophage T5-encoded
  gene A2 protein was determined by protein sequencing. The 134-residue
  sequence is closely similar to that reported for the product of gene
  A2-A3 of bacteriophage BF23. Segments of the sequence are similar to
  segments of bacteriophage T4 gene 32  protein ,  bacteriophage  T3 RNA
  polymerase, and the protein encoded by the host gene responsible for
  isoenzyme conversion of alkaline phosphatase. The similarity of residues
  26-46 to a portion of rabbit  lipopolysaccharide  binding protein is
  possibly relevant to the function of the A2 protein.

REGISTRY NUMBERS: 9001-78-9: ALKALINE PHOSPHATASE
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DESCRIPTORS: ALKALINE PHOSPHATASE MOLECULAR SEQUENCE DATA
DESCRIPTORS:
  MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Enzymology--
    Biochemistry and Molecular Biophysics; Genetics; Physiology
  BIOSYSTEMATIC NAMES: Viruses--Microorganisms
  COMMON TAXONOMIC TERMS: Microorganisms; Viruses
  CHEMICALS & BIOCHEMICALS: ALKALINE PHOSPHATASE
CONCEPT CODES:
  10064 Biochemistry studies - Proteins, peptides and amino acids
  10506 Biophysics - Molecular properties and macromolecules
  10806 Enzymes - Chemical and physical
  31000 Physiology and biochemistry of bacteria
  31500 Genetics of bacteria and viruses
BIOSYSTEMATIC CODES:
  03000 Viruses

...ABSTRACT: bacteriophage BF23. Segments of the sequence are similar to
  segments of bacteriophage T4 gene 32 protein , bacteriophage T3 RNA
  polymerase, and the protein encoded by the host gene responsible for
  isoenzyme conversion of alkaline phosphatase. The similarity of residues
  26-46 to a portion of rabbit lipopolysaccharide binding protein is
  possibly relevant to the function of the A2 protein.

 11/9,K/2   (Item 1 from file: 24)
DIALOG(R)File 24:CSA Life Sciences Abstracts

0001400827   IP ACCESSION NO: 3623578
Amino acid sequence of the bacteriophage T5 gene A2 protein

Snyder, CE Jr
Peptide Synth. Lab., Clin. Immunol. Serv., PRI/DynCorp, NCI-FCRDC,
Frederick, MD 21702, USA

Biochemical and Biophysical Research Communications, v 177, n 3, p
1240-1243, 1991
ADDL. SOURCE INFO: Biochemical and Biophysical Research Communications
[BIOCHEM. BIOPHYS. RES. COMMUN.], vol. 177, no. 3, pp. 1240-1243, 1991
PUBLICATION DATE: 1991

DOCUMENT TYPE: Journal Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English
ISSN: 0006-291X
FILE SEGMENT: Virology & AIDS Abstracts

ABSTRACT:
  The complete amino acid sequence of the bacteriophage T5-encoded gene A2
protein was determined by protein sequencing. The 134-residue sequence is
closely similar to that reported for the product of gene A2-A3 of
bacteriophage BF23. Segments of the sequence are similar to segments of
bacteriophage T4 gene 32 protein , bacteriophage T3 RNA polymerase, and
the protein encoded by the host gene responsible for isoenzyme conversion
of alkaline phosphatase. The similarity of residues 26-46 to a portion of a
rabbit lipopolysaccharide binding protein is possibly relevant to the

function of the A2 protein.

DESCRIPTORS: gene products; amino acid sequence; proteins; homology; phage T5
SUBJ CATG: 22032, Viral proteins

ABSTRACT:
... bacteriophage BF23. Segments of the sequence are similar to segments of bacteriophage T4 gene 32 protein , bacteriophage T3 RNA polymerase, and the protein encoded by the host gene responsible for isoenzyme conversion of alkaline phosphatase. The similarity of residues 26-46 to a portion of a rabbit lipopolysaccharide binding protein is possibly relevant to the function of the A2 protein.

11/9,K/3 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07599434 Genuine Article#: 186NX Number of References: 16
Title: Specific interaction of fused H protein of bacteriophage phi X174 with receptor lipopolysaccharides
Author(s): Suzuki R; Inagaki M; Karita S; Kawaura T; Kato M; Nishikawa S; Kashimura N (REPRINT) ; Morita J
Corporate Source: MIE UNIV,DEPT BIOSCI, 1515 KAMIHAMA/TSU/MIE 5148507/JAPAN/ (REPRINT); MIE UNIV,DEPT BIOSCI/TSU/MIE 5148507/JAPAN/; MIE UNIV,CTR MOL BIOL & GENET/TSU/MIE 5148507/JAPAN/; DOSHISHA WOMENS COLL LIBERAL ARTS,DEPT FOOD SCI & NUTR, KAMIGYO KU/KYOTO 6020893//JAPAN/
Journal: VIRUS RESEARCH, 1999, V60, N1 (MAR), P95-99
ISSN: 0168-1702 Publication date: 19990300
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Geographic Location: JAPAN
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: VIROLOGY
Abstract: The DNA fragment encoding the spike H protein of bacteriophage phi X174 was amplified by polymerase chain reaction. The fragment was sub-cloned into pQE-30 to yield pQE-H. The histidine-tagged H protein (HisH) was obtained from the cell extract of Escherichia coli M15 (pREP4) harboring pQE-H and purified by nickel chelating and anion-exchange chromatographies. HisH was shown to bind dose-dependently to the lipopolysaccharides (LPSs) isolated from phi X174-sensitive strains, E. Coli C or Salmonella typhimuirum TV119 (Ra mutant). In sharp contrast, HisH did not bind to the LPSs from insensitive strains, E. coli F583 (Rd, mutant) or E. coli O111:B4(smooth strain). Since the same selectivity was observed in the plaque counting assay for in vitro inactivation of phi X174, the spike H protein was shown to recognize receptor LPS. (C) 1999 Elsevier Science B.V. All rights reserved.
Descriptors--Author Keywords: spike H protein ; bacteriophage phi X174 ; rough strain lipopolysaccharide ; molecular recognition
Identifiers--KeyWord Plus(R): CELL-WALL LIPOPOLYSACCHARIDE ; ATTACHMENT; ECLIPSE; S13
Cited References:
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...Identifiers--CELL-WALL LIPOPOLYSACCHARIDE ; ATTACHMENT; ECLIPSE; S13

11/9,K/4 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06013714 Genuine Article#: XP101 Number of References: 61
Title: Colicin U, a novel colicin produced by Shigella boydii
Author(s): Smajs D; Pilsl H; Braun V (REPRINT)
Corporate Source: MORGENSTELLE 28,/D-72076 TUBINGEN//GERMANY/ (REPRINT); MASARYK UNIV,FAC MED, DEPT BIOL/BRNO//CZECH REPUBLIC/; UNIV TUBINGEN,/TUBINGEN//GERMANY/
Journal: JOURNAL OF BACTERIOLOGY, 1997, V179, N15 (AUG), P4919-4928
ISSN: 0021-9193 Publication date: 19970800
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
Language: English Document Type: ARTICLE
Geographic Location: GERMANY; CZECH REPUBLIC
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: MICROBIOLOGY
Abstract: A novel colicin, designated colicin U, was found in two Shigella boydii strains of serovars 1 and 8. Colicin U aas active against bacterial strains of the genera Escherichia and Shigella. Plasmid pColU (7.3 kb) of the colicinogenic strain S. boydii M592 (serovar 8) was sequenced, and three colicin genes were identified. The colicin U activity gene, cua, encodes a protein of 619 amino acids (M-r, 66,289); the immunity gene, cui, encodes a protein of 174 amino acids (M-r, 20,688); and the lytic protein gene, cul, encodes a polypeptide of 45 amino acids (M-r, 4,672). Colicin U displays sequence similarities to various colicins. The N-terminal sequence of 130 amino acids has 54% identity to the N-terminal sequence of bacteriocin 28b produced by Serratia marcescens. Furthermore, the N-terminal 36 amino acids have striking sequence identity (83%) to colicin A. Although the C-terminal pore-forming sequence of colicin U shows the highest degree of identity (73%) to the pore-forming C-terminal sequence of colicin B, the immunity protein, which interacts with the same region, displays a higher degree of sequence similarity to the immunity protein of colicin A (45%) than to the immunity protein of colicin B (30.5%), Immunity specificity is probably conferred by a short sequence from residues 571 to residue 599 of colicin U; this sequence is not similar to that of colicin B. We showed that binding of colicin U to sensitive cells is mediated by the OmpA protein, the OmpF porin, and core

lipopolysaccharide . Uptake of colicin U was dependent on the TolA, -B, -Q, and -R proteins. pColU is homologous to plasmid pSB41 (4.1 kb) except for the colicin genes on pColU. pSB41 and pColU coexist in S. boydii strains and can be cotransformed into Escherichia coil, and both plasmids are homologous to pColE1.

Identifiers--KeyWord Plus(R): OUTER-MEMBRANE PROTEIN ; BACTERIOPHAGE -T7 RNA-POLYMERASE; CELL-ENVELOPE MEMBRANE; DNA-SEQUENCE ANALYSIS; TONB-EXBB-EXBD; ESCHERICHIA-COLI; NUCLEOTIDE-SEQUENCE; SERRATIA-MARCESCENS; CROSS-RESISTANCE; STRUCTURAL GENE

Research Fronts: 95-8434 002 (HIGH-LEVEL EXPRESSION OF HETEROLOGOUS GENES; E2 PROTEINS; OPEN READING FRAME)

95-5061 001 (STRUCTURAL GENE; GLTC-DEPENDENT REGULATION OF BACILLUS-SUBTILIS GLUTAMATE SYNTHASE EXPRESSION; ARABIDOPSIS TYPE-1 PROTEIN PHOSPHATASE)

95-6015 001 (LIPOPOLYSACCHARIDE O-ANTIGEN; RFB GENES; BACTERIAL OUTER-MEMBRANE; ALTERED LIPOOLIGOSACCHARIDE EXPRESSION; DIFFERENT SHIGELLA-FLEXNERI SEROTYPES)

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...Abstract: U to sensitive cells is mediated by the OmpA protein, the OmpF porin, and core lipopolysaccharide . Uptake of colicin U was dependent on the TolA, -B, -Q, and -R proteins. pColU...
...Identifiers--OUTER-MEMBRANE PROTEIN ; BACTERIOPHAGE -T7 RNA-POLYMERASE; CELL-ENVELOPE MEMBRANE; DNA-SEQUENCE ANALYSIS; TONB-EXBB-EXBD; ESCHERICHIA-COLI; NUCLEOTIDE...
...Research Fronts: REGULATION OF BACILLUS-SUBTILIS GLUTAMATE SYNTHASE EXPRESSION; ARABIDOPSIS TYPE-1 PROTEIN PHOSPHATASE)
95-6015 001 (LIPOPOLYSACCHARIDE O-ANTIGEN; RFB GENES; BACTERIAL OUTER-MEMBRANE; ALTERED LIPOOLIGOSACCHARIDE EXPRESSION; DIFFERENT SHIGELLA-FLEXNERI SEROTYPES)

11/9,K/5 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

01074762 1999063186
Specific interaction of fused H protein of bacteriophage phiX174 with receptor lipopolysaccharides
Suzuki R.; Inagaki M.; Karita S.; Kawaura T.; Kato M.; Nishikawa S.; Kashimura N.; Morita J.
ADDRESS: N. Kashimura, Department of Bioscience, Mie University, 1515 Kamihama, Tsu, Mie 514-8507, Japan
EMAIL: kasimura@bio.mie-u.ac.jp
Journal: Virus Research, 60/1 (95-99), 1999, Netherlands
CODEN: VIRED
ISSN: 0168-1702
PUBLISHER ITEM IDENTIFIER: S0168170298001452
DOCUMENT TYPE: Short Survey
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 16

The DNA fragment encoding the spike H protein of bacteriophage phiX174 was

amplified by polymerase chain reaction. The fragment was sub-cloned into pQE-30 to yield pQE-H. The histidine-tagged H protein (HisH) was obtained from the cell extract of Escherichia coli M15 (pREP4) harboring pQE-H and purified by nickel chelating and anion-exchange chromatographies. HisH was shown to bind dose-dependently to the lipopolysaccharides (LPSs) isolated from phiX174-sensitive strains, E. coli C or Salmonella typhimurium TV119 (Ra mutant). In sharp contrast, HisH did not bind to the LPSs from insensitive strains, E. coli F583 (Rdinf 2 mutant) or E. coli O111:B4 (smooth strain). Since the same selectivity was observed in the plaque counting assay for in vitro inactivation of phiX174, the spike H protein was shown to recognize receptor LPS. Copyright (C) 1999 Elsevier Science B.V.

DESCRIPTORS:
Spike H protein ; Bacteriophage phiX174; Rough strain lipopolysaccharide ; Molecular recognition

CLASSIFICATION CODE AND DESCRIPTION:
99 - General

DESCRIPTORS:
Spike H protein ; Bacteriophage phiX174; Rough strain lipopolysaccharide ; Molecular recognition

11/9,K/6 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

14215952 EMBASE No: 2006630436
O side chain deficiency enhances sensitivity of Escherichia coli to Shiga toxin 2-converting bacteriophages
Iguchi A.; Iyoda S.; Watanabe H.; Osawa R.
A. Iguchi, Department of Bioscience, Graduate School of Science and Technology, Kobe University, Rokko-dai 1-1, Kobe, Nada-ku 657-8501 Japan

AUTHOR EMAIL: osawa@ans.kobe-u.ac.jp
Current Microbiology (CURR. MICROBIOL.) (United States) 2007, 54/1 (14-19)
CODEN: CUMID ISSN: 0343-8651 eISSN: 1432-0991
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 34

We investigated the relationship between expression of the O side chain of outer membrane lipopolysaccharide (LPS) and infection by a Shiga toxin 2 (Stx2)-converting phage in normal and benign strains of Escherichia coli. Of 19 wild-type E. coli strains isolated from the feces of healthy subjects, those with low-molecular-weight LPS showed markedly higher susceptibility to lytic and lysogenic infection by Stx2 phages than those with high-molecular-weight LPS. All lysogens produced infectious phage particles and Stx2. The Stx-negative E. coli O157:H7 strain ATCC43888 with an intact O side chain was found to be resistant to lysis by an Stx2 phage and lysogenic infection by a recombinant Stx2 phage, whereas a rfbE mutant deficient in the expression of the O side chain was readily infected by the phage and yielded stable lysogens. The evidence suggests that an O side chain deficiency leads to the creation of new pathotypes of Shiga

toxin-producing E. coli (STEC) within the intestinal microflora. (c) 2006 Springer Science+Business Media, Inc.

DRUG DESCRIPTORS:
*O antigen--endogenous compound--ec
mutant protein; verotoxin 2
MEDICAL DESCRIPTORS:
*Escherichia coli; *Shiga toxin producing Escherichia coli; * bacteriophage ; * protein deficiency
article; bacterial strain; bacterium isolation; feces; gene expression; infection sensitivity; intestine flora; lysis; lysogenization; molecular weight; nonhuman; pathotype; priority journal; wild type
SECTION HEADINGS:
  004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
  We investigated the relationship between expression of the O side chain of outer membrane lipopolysaccharide (LPS) and infection by a Shiga toxin 2 (Stx2)-converting phage in normal and benign...
MEDICAL DESCRIPTORS:
*Escherichia coli; *Shiga toxin producing Escherichia coli; * bacteriophage ; * protein deficiency

11/9,K/7 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

12016512 EMBASE No: 2003127822
  Sequence and structural diversity in endotoxin-binding dodecapeptides
  Zhu Y.; Ho B.; Ding J.L.
  J.L. Ding, Marine Biotechnology Laboratory, Department of Biological Sciences, National University of Singapore, 10 Kent Ridge Cresent, Singapore 117543 Singapore
  AUTHOR EMAIL: dbsdjl@nus.edu.sg
  Biochimica et Biophysica Acta - Biomembranes (BIOCHIM. BIOPHYS. ACTA BIOMEMBR.) (Netherlands) 01 APR 2003, 1611/1-2 (234-242)
  CODEN: BBBMB ISSN: 0005-2736
  DOCUMENT TYPE: Journal ; Article
  LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
  NUMBER OF REFERENCES: 47

  For the study of sequence or structure requirement of short peptides for endotoxin binding, and to search for potential endotoxin antagonists, biopanning was carried out on a phage-displayed random dodecapeptide library against immobilized lipopolysaccharide (LPS) or lipid A (LA), the core toxic portion of LPS. Specific binding of selected phage-displayed peptides to LPS/LA was confirmed by surface plasmon resonance (SPR) analysis. These peptides are rich in basic and hydrophobic amino acids, especially histidine, proline and tryptophan, highlighting apparent amphiphilicity and bacterial membrane activity. These dodecapeptide sequences have no predictable secondary structure in solution, indicating the importance of a random structure before their interaction with LPS/LA. Sequence alignment reveals various potential secondary structures with these selected peptides, which contain specific signature motifs such as b(p)hb(p)hb(p), bbbb, hhhh (b - basic, p - polar, h - hydrophobic residue), capable of binding LPS/LA. However, none of these peptides exhibit a significant calculated structural amphiphilicity while assuming a secondary structure. This study suggests that for these short dodecapeptides to bind

LPS/LA, the potential for their structural adaptation is more important than an amphipathic structure. (c) 2003 Elsevier Science B.V. All rights reserved.

DRUG DESCRIPTORS:
*endotoxin; *peptide derivative
lipopolysaccharide ; tryptophan; unclassified drug
MEDICAL DESCRIPTORS:
protein structure; bacteriophage ; protein binding ; surface plasmon resonance; hydrophobicity; bacterial membrane; amino acid sequence; protein motif; article; priority journal
DRUG TERMS (UNCONTROLLED): dodecapeptide
CAS REGISTRY NO.: 6912-86-3, 73-22-3 (tryptophan)
SECTION HEADINGS:
029 Clinical and Experimental Biochemistry

...endotoxin antagonists, biopanning was carried out on a phage-displayed random dodecapeptide library against immobilized lipopolysaccharide (LPS) or lipid A (LA), the core toxic portion of LPS. Specific binding of selected...
DRUG DESCRIPTORS:
lipopolysaccharide ; tryptophan; unclassified drug
MEDICAL DESCRIPTORS:
protein structure; bacteriophage ; protein binding ; surface plasmon resonance; hydrophobicity; bacterial membrane; amino acid sequence; protein motif; article; priority journal

11/9,K/8 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE

04778507 EMBASE No: 1991273243
Amino acid sequence of the bacteriophage T5 gene A2 protein
Snyder Jr. C.E.
Peptide Synthesis Laboratory, Clinical Immunology Services, PRI/DynCorp, NCI-FCRDC,Frederick, MD 21702 United States
Biochemical and Biophysical Research Communications (BIOCHEM. BIOPHYS. RES. COMMUN.) (United States) 1991, 177/3 (1240-1246)
CODEN: BBRCA ISSN: 0006-291X
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The complete amino acid sequence of the bacteriophage T5-encoded gene A2 protein was determined by protein sequencing. The 134-residue sequence is closely similar to that reported for the product of gene A2-A3 of bacteriophage BF23. Segments of the sequence are similar to segments of bacteriophage T4 gene 32 protein , bacteriophage T3 RNA polymerase, and the protein encoded by the host gene responsible for isoenzyme conversion of alkaline phosphatase. The similarity of residues 26-46 to a portion of a rabbit lipopolysaccharide binding protein is possibly relevant to the function of the A2 protein.
DRUG DESCRIPTORS:
*virus protein--endogenous compound--ec
MEDICAL DESCRIPTORS:
*amino acid sequence; *bacteriophage t5
article; nonhuman; priority journal

SECTION HEADINGS:
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology

...bacteriophage BF23. Segments of the sequence are similar to segments of bacteriophage T4 gene 32 protein , bacteriophage T3 RNA polymerase, and the protein encoded by the host gene responsible for isoenzyme conversion of alkaline phosphatase. The similarity of residues 26-46 to a portion of a rabbit lipopolysaccharide binding protein is possibly relevant to the function of the A2 protein.

11/9,K/9 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

10475154 PASCAL No.: 92-0678648
Amino acid sequence of the bacteriophage T5 gene A2 protein
SNYDER C E JR
PRI/DynCorp, peptide synthesis lab., clin. immunology serv., Frederick MD 21702, USA
Journal: Biochemical and biophysical research communications, 1991, 177 (3) 1240-1246
ISSN: 0006-291X CODEN: BBRCA9 Availability: INIST-8252; 354000011808510530
No. of Refs.: 26 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: USA
Language: English Summary Language: English

The complete amino acid sequence of the bacteriophage T5-encoded gene A2 protein was determined by protein sequencing. The 134-residue sequence is closely similar to that reported for the product of gene A2-A3 of bacteriophage BF23. Segments of the sequence are similar to segments of bacteriophage T4 gene 32 protein , bacteriophage T3 RNA polymerase, and the protein encoded by the host gene responsible for isoenzyme conversion of alkaline phosphatase. The similarity of residues 26-46 to a portion of a rabbit lipopolysaccharide binding protein is possibly relevant to the function of the A2 protein.

English Descriptors: Binding protein; Phage T5; Aminoacid sequence; Homology; Enzymatic digestion; Computer aid
Broad Descriptors: Siphoviridae; Phage; Virus; Siphoviridae; Bacteriophage; Virus; Siphoviridae; Phage; Virus

French Descriptors: Proteine liaison; Bacteriophage T5; Sequence aminoacide ; Homologie; Degradation enzymatique; Assistance ordinateur; Gene A2

Classification Codes: 002A02D08

... bacteriophage BF23. Segments of the sequence are similar to segments of bacteriophage T4 gene 32 protein , bacteriophage T3 RNA polymerase, and the protein encoded by the host gene responsible for isoenzyme conversion of alkaline phosphatase. The similarity of residues 26-46 to a portion of a rabbit lipopolysaccharide binding protein is possibly relevant to the function of the A2 protein.

11/9,K/10 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

19786424 PMID: 16096708
Conservation of the N-terminus of some phage tail proteins.
Villafane R; Costa S; Ahmed R; Salgado C
Department of Biochemistry Ponce School of Medicine, Ponce, Puerto Rico. drbob@psm.edu
Archives of virology (Austria) Dec 2005, 150 (12) p2609-21, ISSN 0304-8608--Print Journal Code: 7506870
Contract/Grant No.: 2G12 RR003050; RR; NCRR; S06 GM50695-04; GM; NIGMS
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
To study the interaction between lipopolysaccharide and protein, a comparative approach was employed using seven Salmonella enterica serovar Typhimurium typing phages as the protein model systems. This interaction has been studied in detail in the Salmonella enterica serovar Typhimurium phage P22 system and involves only the viral tailspike protein. Similarity between these phages and phage P22 was monitored in this Report by assaying restriction endonuclease digestions, capsid size, reactivity to the P22 tailspike protein monoclonal antibody, mAb92, which reacts with the N-terminus of the P22 tail protein and the ability to produce a PCR fragment using primers made to the ends of the P22 tailspike gene. The data indicate that tailspike similarity exists between most of these phages and a scheme reclassifying them is presented and that the N-terminus of the P22 tailspike protein may be a motif for many phage systems and may serve as a aid in the taxonomy of phages. The data suggest a classification scheme in which the N-terminus of some tailspike proteins (head-binding region in some tail proteins) may play a critical element role in the classification of Salmonella viruses.
Descriptors: *Conserved Sequence; *Salmonella Phages--genetics--GE; *Viral Tail Proteins--genetics--GE; Antibodies, Monoclonal--immunology--IM; Antibodies, Viral--immunology--IM; Bacteriophage P22--genetics--GE; Bacteriophage P22--immunology--IM; Blotting, Western; DNA Fingerprinting; DNA, Viral--analysis--AN; Polymerase Chain Reaction; Research Support, N.I.H., Extramural; Salmonella Phages--classification--CL; Salmonella Phages--immunology--IM; Salmonella typhi--virology--VI; Viral Tail Proteins --immunology--IM
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Antibodies, Viral); 0 (DNA, Viral); 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage)
Record Date Created: 20051118
Record Date Completed: 20060125
Date of Electronic Publication: 20050812

To study the interaction between lipopolysaccharide and protein, a comparative approach was employed using seven Salmonella enterica serovar Typhimurium typing phages...
Chemical Name: Antibodies, Monoclonal; Antibodies, Viral; DNA, Viral; Viral Tail Proteins; tailspike protein , bacteriophage
? t s11/9,k/11-19

11/9,K/11 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14952006 PMID: 15215687

Homology between two different Salmonella phages: Salmonella enterica serovar Typhimurium phage P22 and Salmonella enterica serovar Anatum var. 15 + phageepsilon34.

Salgado Clari J; Zayas Milka; Villafane Robert
UCC School of Medicine, Bayamon, Puerto Rico 00960.

Virus genes (United States) Aug 2004, 29 (1) p87-98, ISSN 0920-8569 --Print Journal Code: 8803967
Contract/Grant No.: BRIN PR P20 RR16470; RR; NCRR; MBRS S06 GM50695-04; MB; BHP; MBRS SO6 GM008239; MB; BHP; RCMI G12 RR03050; RC; CCR
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

A distinguishing feature of many microorganisms, belonging to the Gram negative group of bacteria, is the presence of the lipopolysaccharide on their cell surface. Salmonella is a prominent member of this group of bacteria. Many Salmonella phages use the LPS as the initial receptor in the infection process and they can distinguish subtle changes in the LPS molecules. The phage protein that is responsible for recognition of these cells is the tail or tailspike protein (TSP). Those TSPs, which use LPS as a receptor, are prokaryotic LPS-binding proteins. As an initial step in using phage TSPs as model systems for a detailed molecular genetic analysis of protein-LPS interactions, a comparison of two phages and their TSPs from two different Salmonella bacterial viruses (phages), Salmonella enterica serovar Typhimurium phage P22 and Salmonella enterica serovar Anatum var. 15 + phage epsilon34, is being carried out. This present study shows significant viral protein homology between many viral structural proteins from these two phages including their TSPs. Significantly this report suggests a general structural motif for part of the TSP of phages and suggests that a more detailed comparative analysis of these TSPs is warranted.

Descriptors: *Bacteriophage P22--genetics--GE; *Salmonella Phages --genetics--GE; *Salmonella enterica--virology--VI; *Salmonella typhimurium --virology--VI; Animals; Bacteriophage P22--metabolism--ME; Bacteriophage Typing; Comparative Study; Evolution, Molecular; Lipopolysaccharides --metabolism--ME; Mice; Research Support, U.S. Gov't, P.H.S.; Salmonella Infections, Animal--virology--VI; Salmonella Phages--metabolism--ME; Viral Structural Proteins--genetics--GE; Viral Structural Proteins--metabolism --ME; Viral Tail Proteins--genetics--GE; Viral Tail Proteins--metabolism --ME
CAS Registry No.: 0 (Lipopolysaccharides); 0 (Viral Structural Proteins); 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage)
Record Date Created: 20040624
Record Date Completed: 20041021

...many microorganisms, belonging to the Gram negative group of bacteria, is the presence of the lipopolysaccharide on their cell surface. Salmonella is a prominent member of this group of bacteria. Many...

Chemical Name: Lipopolysaccharides; Viral Structural Proteins; Viral Tail Proteins; tailspike protein , bacteriophage

11/9,K/12 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13705615 PMID: 11955057
Characterization of the protrimer intermediate in the folding pathway of the interdigitated beta-helix tailspike protein.
Benton Christopher B; King Jonathan; Clark Patricia L
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.
Biochemistry (United States) Apr 23 2002, 41 (16) p5093-103, ISSN 0006-2960--Print Journal Code: 0370623
Contract/Grant No.: GM17980; GM; NIGMS; GM19715; GM; NIGMS
Publishing Model Print; Erratum in Biochemistry 2002 Aug 20;41(33) 10570
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
P22 tailspike is a homotrimeric, thermostable adhesin that recognizes the O-antigen lipopolysaccharide of Salmonella typhimurium. The 70 kDa subunits include long beta-helix domains. After residue 540, the polypeptide chains change their path and wrap around one another, with extensive interchain contacts. Formation of this interdigitated domain intimately couples the chain folding and assembly mechanisms. The earliest detectable trimeric intermediate in the tailspike folding and assembly pathway is the protrimer, suspected to be a precursor of the native trimer structure. We have directly analyzed the kinetics of in vitro protrimer formation and disappearance for wild type and mutant tailspike proteins. The results confirm that the protrimer intermediate is an on-pathway intermediate for tailspike folding. Protrimer was originally resolved during tailspike folding because its migration through nondenaturing polyacrylamide gels was significantly retarded with respect to the migration of the native tailspike trimer. By comparing protein mobility versus acrylamide concentration, we find that the retarded mobility of the protrimer is due exclusively to a larger overall size than the native trimer, rather than an altered net surface charge. Experiments with mutant tailspike proteins indicate that the conformation difference between protrimer and native tailspike trimer is localized toward the C-termini of the tailspike polypeptide chains. These results suggest that the transformation of the protrimer to the native tailspike trimer represents the C-terminal interdigitation of the three polypeptide chains. This late step may confer the detergent-resistance, protease-resistance, and thermostability of the native trimer.
Descriptors: *Bacteriophage P22--enzymology--EN; *Glycoside Hydrolases --chemistry--CH; *Glycoside Hydrolases--metabolism--ME; *Protein Folding; *Viral Tail Proteins--chemistry--CH; *Viral Tail Proteins--metabolism--ME ; Amino Acid Substitution--genetics--GE; Arginine--genetics--GE; Bacteriophage P22--genetics--GE; Electrophoresis, Polyacrylamide Gel; Evolution, Molecular; Glutathione--pharmacology--PD; Glycine--genetics--GE; Glycoside Hydrolases--biosynthesis--BI; Glycoside Hydrolases--genetics--GE ; Kinetics; Oxidation-Reduction; Point Mutation; Protein Conformation; Protein Structure, Secondary--drug effects--DE; Protein Structure, Secondary--genetics--GE; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.; Salmonella typhimurium--virology--VI; Surface

Properties; Temperature; Viral Tail Proteins--biosynthesis--BI; Viral Tail Proteins--genetics--GE
CAS Registry No.: 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage); 56-40-6 (Glycine); 70-18-8 (Glutathione); 74-79-3 (Arginine)
Enzyme No.: EC 3.2.1. (Glycoside Hydrolases)
Record Date Created: 20020416
Record Date Completed: 20020509

P22 tailspike is a homotrimeric, thermostable adhesin that recognizes the O-antigen lipopolysaccharide of Salmonella typhimurium. The 70 kDa subunits include long beta-helix domains. After residue 540...
Chemical Name: Viral Tail Proteins; tailspike protein , bacteriophage ; Glycine; Glutathione; Arginine; Glycoside Hydrolases

11/9,K/13 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12494062 PMID: 10439404
The Shigella flexneri bacteriophage Sf6 tailspike protein (TSP)/endorhamnosidase is related to the bacteriophage P22 TSP and has a motif common to exo- and endoglycanases, and C-5 epimerases.
Chua J E; Manning P A; Morona R
Department of Microbiology and Immunology, University of Adelaide, South Australia.
Microbiology (Reading, England) (ENGLAND) Jul 1999, 145 (Pt 7) p1649-59, ISSN 1350-0872--Print Journal Code: 9430468
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The temperate bacteriophage Sf6 infects Shigella flexneri strains of serotype X or Y, converting them into serotypes 3a or 3b, respectively. The tailspike protein (TSP) of Sf6 possesses endo-1,3-alpha-L-rhamnosidase (endorhamnosidase) activity which results in cleavage of the lipopolysaccharide O-antigen receptor during the adsorption of the phage to the cell surface. When used in Southern hybridization, a P22 gene 9 (encoding P22 TSP) DNA probe hybridized with restriction fragment PstI-7 of Sf6. DNA sequencing and analysis of PstI-7 and the adjacent PstI-8 fragment revealed an open reading frame (ORF1) of 1872 bp (624 amino acids) bearing amino acid sequence homology to the bacteriophage P22 TSP N-terminal head-binding domain. High conservation of key residues was suggestive of similar secondary and tertiary N-terminal protein structure and a similar function of the Sf6 TSP in this region. In addition, an amino acid sequence motif (DFGX3DGX6AX3A) was identified between residues 164 and 184 which was also found to exist in various prokaryotic and eukaryotic exo-/endoglycanases, C-5 epimerases and bacteriophage proteins. Expression of ORF1 from a T7 promoter produced a 67 kDa protein (detected by L-[35S]methionine labelling and SDS-PAGE). Assay of heat-treated cytoplasmic extracts containing the ORF1-encoded protein by incubation with whole Sh. flexneri Y cells demonstrated that O-antigen hydrolysis activity was present; ORF1 therefore encodes Sf6 TSP. Sf6 TSP exhibited specific and preferential activity for long-chain Sh. flexneri serotype X or Y

O-antigen, cleavage of which resulted in the release of oligosaccharide fragments, consistent with octasaccharides in size, as detected by fluorophore-assisted carbohydrate electrophoresis (FACE).

Descriptors: *Bacteriophages--enzymology--EN; *Glycoside Hydrolases --chemistry--CH; *Shigella flexneri--virology--VI; *Viral Tail Proteins --chemistry--CH; Amino Acid Sequence; Base Sequence; Blotting, Southern; Carbohydrate Sequence; Cloning, Molecular; DNA, Viral--analysis--AN; Deoxyribonucleases, Type II Site-Specific--metabolism--ME; Electrophoresis, Polyacrylamide Gel; Glycoside Hydrolases--genetics--GE; Glycoside Hydrolases--metabolism--ME; Molecular Sequence Data; Polymerase Chain Reaction; Research Support, Non-U.S. Gov't; Restriction Mapping; Sequence Analysis, DNA; Viral Tail Proteins--genetics--GE; Viral Tail Proteins --metabolism--ME

Molecular Sequence Databank No.: GENBANK/AF128887

CAS Registry No.: 0 (DNA, Viral); 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage)

Enzyme No.: EC 3.1.21.- (endodeoxyribonuclease PstI); EC 3.1.21.4 (Deoxyribonucleases, Type II Site-Specific); EC 3.2.1. (Glycoside Hydrolases); EC 3.2.1.- (endorhamnosidase)

Record Date Created: 19991028

Record Date Completed: 19991028

... possesses endo-1,3-alpha-L-rhamnosidase (endorhamnosidase) activity which results in cleavage of the lipopolysaccharide O-antigen receptor during the adsorption of the phage to the cell surface. When used...

Chemical Name: DNA, Viral; Viral Tail Proteins; tailspike protein , bacteriophage ; endodeoxyribonuclease PstI; Deoxyribonucleases, Type II Site-Specific; Glycoside Hydrolases; endorhamnosidase

11/9,K/14 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12104340 PMID: 10543960

Mutations improving the folding of phage P22 tailspike protein affect its receptor binding activity.

Baxa U; Steinbacher S; Weintraub A; Huber R; Seckler R

Physikalische Biochemie, Universitat Potsdam, Im Biotechnologiepark, Luckenwalde, D-14943, Germany.

Journal of molecular biology (ENGLAND) Oct 29 1999, 293 (3) p693-701 , ISSN 0022-2836--Print Journal Code: 2985088R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Four previously isolated mutations in Salmonella phage P22 tailspike protein were used to study the relationship between protein stability, folding, and function. Tailspike protein binds and hydrolyzes the repetitive O-antigen structure in Salmonella lipopolysaccharide . Four mutations (V331G, V331A, A334V, A334I) are known to increase the folding efficiency, and two of them (at position 331) also increase the thermal stability of the protein. Octasaccharides comprising two repeating units of the O-antigens from two different Salmonella strains were employed to analyze the receptor binding function of the mutant proteins. Their

endorhamnosidase enzymatic activity was assayed with the aid of a fluorescence-labeled dodecasaccharide. Both V331A and V331G were found to strongly affect O-antigen binding. Octasaccharide binding affinities of the mutant proteins are reduced tenfold and 200-fold, corresponding to a loss of 17% and 36% of the standard free energy of binding, respectively. Both mutations at position 334 affected O-antigen binding only slightly (DeltaDeltaG(0)B approximately 1 kJ/mol), but these mutations reduce the thermal stability of the protein. The observed effects on the endoglycosidase activity are fully explained by the changes in substrate binding, suggesting that neither of the mutations affect the catalytic rate. Crystal structures of all four mutants were determined to a resolution of 2.0 A. Except for the partly or completely missing side-chain, no significant changes compared to the wild-type protein structure were found for the mutants at position 331, whereas a small but significant backbone displacement around the mutation site in A334V and A334I may explain the observed thermal destabilization. Copyright 1999 Academic Press.

Descriptors: *Bacteriophage P22--enzymology--EN; *Glycoside Hydrolases --chemistry--CH; *Glycoside Hydrolases--metabolism--ME; *Lipopolysaccharides--metabolism--ME; *Mutation; *Protein Folding; *Viral Tail Proteins --chemistry--CH; *Viral Tail Proteins--metabolism--ME; Bacteriophage P22 --genetics--GE; Bacteriophage P22--metabolism--ME; Crystallization; Crystallography, X-Ray; Enzyme Stability; Fluorescence; Glycoside Hydrolases--genetics--GE; Hydrolysis; Kinetics; Lipopolysaccharides --chemistry--CH; Models, Molecular; Molecular Sequence Data; Oligosaccharides--metabolism--ME; Protein Structure, Secondary; Research Support, Non-U.S. Gov't; Salmonella--metabolism--ME; Salmonella--virology --VI; Structure-Activity Relationship; Thermodynamics; Titrimetry; Viral Tail Proteins--genetics--GE

Molecular Sequence Databank No.: PDB/1CLW; PDB/1QA1; PDB/1QA2; PDB/1QA3; PDB/1TSP

CAS Registry No.: 0 (Lipopolysaccharides); 0 (Oligosaccharides); 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage)

Enzyme No.: EC 3.2.1. (Glycoside Hydrolases)

Record Date Created: 19991119

Record Date Completed: 19991119

... folding, and function. Tailspike protein binds and hydrolyzes the repetitive O-antigen structure in Salmonella lipopolysaccharide . Four mutations (V331G, V331A, A334V, A334I) are known to increase the folding efficiency, and two...

Chemical Name: Lipopolysaccharides; Oligosaccharides; Viral Tail Proteins ; tailspike protein , bacteriophage ; Glycoside Hydrolases

11/9,K/15 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11898217 PMID: 9724623

Folding and function of repetitive structure in the homotrimeric phage P22 tailspike protein.

Seckler R

Institut fur Biophysik und Physikalische Biochemie, Regensburg, D-93040, Germany.

Journal of structural biology (UNITED STATES) 1998, 122 (1-2) p216-22, ISSN 1047-8477--Print Journal Code: 9011206

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The Salmonella bacteriophage P22 recognizes its host cell receptor, lipopolysaccharide , by means of six tailspikes, thermostable homotrimers of 72-kDa polypeptides. Biophysical results on the binding reaction, together with high-resolution structural information from X-ray crystallography, have shed light on the interactions determining the viral host range. Folding and assembly of the tailspike protein in vitro have been analyzed in detail, and the data have been compared with observations on the in vivo assembly pathway. Repetitive structural elements in the tailspike protein, like a side-by-side trimer of parallel beta-helices, a parallel alpha-helical bundle, a triangular prism made up from antiparallel beta-sheets, and a short segment of a triple beta-helix can be considered building blocks for larger structural proteins, and thus, the results on P22 tailspike may have implications for fibrous protein structure and folding. Copyright 1998 Academic Press.

Descriptors: *Bacteriophage P22--chemistry--CH; *Glycoside Hydrolases --chemistry--CH; *Protein Folding; *Viral Tail Proteins--chemistry--CH; Carbohydrate Sequence; Crystallography, X-Ray; Models, Molecular; Molecular Sequence Data; Protein Conformation; Protein Structure, Secondary; Research Support, Non-U.S. Gov't

CAS Registry No.: 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage)

Enzyme No.: EC 3.2.1. (Glycoside Hydrolases)

Record Date Created: 19981029

Record Date Completed: 19981029

The Salmonella bacteriophage P22 recognizes its host cell receptor, lipopolysaccharide , by means of six tailspikes, thermostable homotrimers of 72-kDa polypeptides. Biophysical results on the...

Chemical Name: Viral Tail Proteins; tailspike protein , bacteriophage ; Glycoside Hydrolases

11/9,K/16 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11321798 PMID: 9135118

Phage P22 tailspike protein: crystal structure of the head-binding domain at 2.3 A, fully refined structure of the endorhamnosidase at 1.56 A resolution, and the molecular basis of O-antigen recognition and cleavage.

Steinbacher S; Miller S; Baxa U; Budisa N; Weintraub A; Seckler R; Huber R

Max-Planck-Institut fur Biochemie, Abteilung fur Strukturforschung, Martinsried, Germany.

Journal of molecular biology (ENGLAND) Apr 11 1997, 267 (4) p865-80, ISSN 0022-2836--Print Journal Code: 2985088R

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The tailspike protein of Salmonella phage P22 is a viral adhesion protein with both receptor binding and destroying activities. It recognises the O-antigenic repeating units of cell surface lipopolysaccharide of serogroup A, B and D1 as receptor, but also inactivates its receptor by endoglycosidase (endorhamnosidase) activity. In the final step of bacteriophage P22 assembly six homotrimeric tailspike molecules are non-covalently attached to the DNA injection apparatus, mediated by their N-terminal, head-binding domains. We report the crystal structure of the head-binding domain of P22 tailspike protein at 2.3 A resolution, solved with a recombinant telluromethionine derivative and non-crystallographic symmetry averaging. The trimeric dome-like structure is formed by two perpendicular beta-sheets of five and three strands, respectively in each subunit and caps a three-helix bundle observed in the structure of the C-terminal receptor binding and cleaving fragment, reported here after full refinement at 1.56 A resolution. In the central part of the receptor binding fragment, three parallel beta-helices of 13 complete turns are associated side-by-side, while the three polypeptide strands merge into a single domain towards their C termini, with close interdigitation at the junction to the beta-helix part. Complex structures with receptor fragments from S. typhimurium, S. enteritidis and S. typhi253Ty determined at 1.8 A resolution are described in detail. Insertions into the beta-helix form the O-antigen binding groove, which also harbours the active site residues Asp392, Asp395 and Glu359. In the intact structure of the tailspike protein, head-binding and receptor-binding parts are probably linked by a flexible hinge whose function may be either to deal with shearing forces on the exposed, 150 A long tailspikes or to allow them to bend during the infection process.

Descriptors: *Bacteriophage P22--chemistry--CH; *Glycoside Hydrolases --chemistry--CH; *O Antigens--metabolism--ME; *Viral Tail Proteins --chemistry--CH; Amino Acid Sequence; Bacteriophage P22--enzymology--EN; Binding Sites; Carbohydrate Sequence; Crystallography, X-Ray; Glycoside Hydrolases--metabolism--ME; Methionine--analogs and derivatives--AA; Methionine--chemistry--CH; Models, Molecular; Molecular Sequence Data; Protein Conformation; Receptors, Virus--chemistry--CH; Receptors, Virus --metabolism--ME; Recombinant Proteins--chemistry--CH; Research Support, Non-U.S. Gov't; Salmonella--chemistry--CH; Salmonella--virology--VI; Tellurium--chemistry--CH; Viral Tail Proteins--metabolism--ME

CAS Registry No.: 0 (O Antigens); 0 (Receptors, Virus); 0 (Recombinant Proteins); 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage); 0 (telluromethionine); 13494-80-9 (Tellurium); 63-68-3 (Methionine)

Enzyme No.: EC 3.2.1. (Glycoside Hydrolases); EC 3.2.1.- (endorhamnosidase)

Record Date Created: 19970528

Record Date Completed: 19970528

... receptor binding and destroying activities. It recognises the O-antigenic repeating units of cell surface lipopolysaccharide of serogroup A, B and D1 as receptor, but also inactivates its receptor by endoglycosidase...

Chemical Name: O Antigens; Receptors, Virus; Recombinant Proteins; Viral Tail Proteins; tailspike protein , bacteriophage ; telluromethionine; Tellurium; Methionine; Glycoside Hydrolases; endorhamnosidase

11/9,K/17 (Item 8 from file: 155)

11295519 PMID: 9096209

Prevalence of temperature sensitive folding mutations in the parallel beta coil domain of the phage P22 tailspike endorhamnosidase.

Haase-Pettingell C; King J
Department of Biology, MIT, Cambridge, MA 02139, USA.

Journal of molecular biology (ENGLAND) Mar 21 1997, 267 (1) p88-102, ISSN 0022-2836--Print Journal Code: 2985088R
Contract/Grant No.: 17.980; PHS
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Temperature sensitive mutations fall into two general classes: tl mutations, which render the mature protein thermolabile, and tsf (temperature sensitive folding) mutations, which destabilize an intermediate in the folding pathway without altering the functions of the folded state. The molecular defects caused by tsf mutations have been intensively studied for the elongated tailspike endorhamnosidase of Salmonella phage P22. The tailspike, responsible for host cell recognition and attachment, contains a 13 strand parallel beta coil domain. A set of tsf mutants located in the beta coil domain have been shown to cause folding defects in the in vivo folding pathway for the tailspike. We report here additional data on 17 other temperature sensitive mutants which are in the beta coil domain. Using mutant proteins formed at low temperature, the essential functions of assembling on the phage head, and binding to the O-antigen lipopolysaccharide (LPS) receptor of Salmonella were examined at high temperatures. All of the mutant proteins once folded at permissive temperature, were functional at restrictive temperatures. When synthesized at restrictive temperature the mutant chains formed an early folding intermediate, but failed to reach the mature conformation, accumulating instead in the aggregated inclusion body state. Thus this set of mutants all have the temperature sensitive folding phenotype. The prevalence of tsf mutants in the parallel beta coil domain presumably reflects properties of its folding intermediates. The key property may be the tendency of the intermediate to associate off pathway to the kinetically trapped inclusion body state.

Descriptors: *Bacteriophage P22--metabolism--ME; *Glycoside Hydrolases --metabolism--ME; *Mutation; *Protein Folding; *Viral Tail Proteins --metabolism--ME; Glycoside Hydrolases--genetics--GE; Heat; Protein Binding ; Research Support, U.S. Gov't, P.H.S.; Viral Tail Proteins--genetics--GE
CAS Registry No.: 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage)
Enzyme No.: EC 3.2.1. (Glycoside Hydrolases)
Record Date Created: 19970430
Record Date Completed: 19970430

... the essential functions of assembling on the phage head, and binding to the O-antigen lipopolysaccharide (LPS) receptor of Salmonella were examined at high temperatures. All of the mutant proteins once...

Chemical Name: Viral Tail Proteins; tailspike protein , bacteriophage ; Glycoside Hydrolases

11/9,K/18 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11065627 PMID: 8889178
Interactions of phage P22 tails with their cellular receptor, Salmonella O-antigen polysaccharide.
Baxa U; Steinbacher S; Miller S; Weintraub A; Huber R; Seckler R
Universtat Regensburg, Institut fur Biophysik und Physikalische Biochemie, Germany.
Biophysical journal (UNITED STATES) Oct 1996, 71 (4) p2040-8, ISSN 0006-3495--Print Journal Code: 0370626
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
Bacteriophage P22 binds to its cell surface receptor, the repetitive O-antigen structure in Salmonella lipopolysaccharide , by its six homotrimeric tailspikes. Receptor binding by soluble tailspikes and the receptor-inactivating endorhamnosidase activity of the tailspike protein were studied using octa- and dodecasaccharides comprising two and three O-antigen repeats of Salmonella enteritidis and Salmonella typhimurium lipopolysaccharides. Wild-type tailspike protein and three mutants (D392N, D395N, and E359Q) with defective endorhamnosidase activity were used. Oligosaccharide binding to all three subunits, measured by a tryptophan fluorescence quench or by fluorescence depolarization of a coumarin label attached to the reducing end of the dodecasaccharide, occurs independently. At 10 degrees C, the binding affinities of all four proteins to oligosaccharides from both bacterial strains are identical within experimental error, and the binding constants for octa- and dodecasaccharides are 1 x 10(6) M(-1) and 2 x 10(6) M(-1), proving that two O-antigen repeats are sufficient for lipopolysaccharide recognition by the tailspike. Equilibration with the oligosaccharides occurs rapidly, but the endorhamnosidase produces only one cleavage every 100 s at 10 degrees C or about 2 min(-1) at the bacterial growth temperature. Thus, movement of virions in the lipopolysaccharide layer before DNA injection may involve the release and rebinding of individual tailspikes rather than hydrolysis of the O-antigen.
Descriptors: *Bacteriophage P22--physiology--PH; *Glycoside Hydrolases --chemistry--CH; *O Antigens--chemistry--CH; *O Antigens--metabolism--ME; *Protein Conformation; *Salmonella enteritidis--virology--VI; *Salmonella typhimurium--virology--VI; *Viral Tail Proteins--chemistry--CH; Carbohydrate Conformation; Carbohydrate Sequence; Crystallography, X-Ray; Glycoside Hydrolases--metabolism--ME; Models, Molecular; Molecular Sequence Data; Mutagenesis, Site-Directed; Oligosaccharides--chemistry--CH; Recombinant Proteins--chemistry--CH; Recombinant Proteins--metabolism--ME; Research Support, Non-U.S. Gov't; Spectrometry, Fluorescence; Thermodynamics; Viral Tail Proteins--metabolism--ME
CAS Registry No.: 0 (O Antigens); 0 (Oligosaccharides); 0 (Recombinant Proteins); 0 (Viral Tail Proteins); 0 (tailspike protein, bacteriophage)
Enzyme No.: EC 3.2.1. (Glycoside Hydrolases); EC 3.2.1.- (endorhamnosidase)
Record Date Created: 19970211

Record Date Completed: 19970211

Bacteriophage P22 binds to its cell surface receptor, the repetitive O-antigen structure in Salmonella lipopolysaccharide , by its six homotrimeric tailspikes. Receptor binding by soluble tailspikes and the receptor-inactivating endorhamnosidase...

...2 x 10(6) M(-1), proving that two O-antigen repeats are sufficient for lipopolysaccharide recognition by the tailspike. Equilibration with the oligosaccharides occurs rapidly, but the endorhamnosidase produces only...

... about 2 min(-1) at the bacterial growth temperature. Thus, movement of virions in the lipopolysaccharide layer before DNA injection may involve the release and rebinding of individual tailspikes rather than...

Chemical Name: O Antigens; Oligosaccharides; Recombinant Proteins; Viral Tail Proteins; tailspike protein , bacteriophage ; Glycoside Hydrolases; endorhamnosidase

11/9,K/19 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08865813 PMID: 2059212

Amino acid sequence of the bacteriophage T5 gene A2 protein.

Snyder C E

Peptide Synthesis Laboratory, PRI/DynCorp, NCI-FCRDC, Frederick, Maryland 21702.

Biochemical and biophysical research communications (UNITED STATES) Jun 28 1991, 177 (3) p1240-6, ISSN 0006-291X--Print Journal Code: 0372516

Contract/Grant No.: N01-C0-74102; PHS

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The complete amino acid sequence of the bacteriophage T5-encoded gene A2 protein was determined by protein sequencing. The 134-residue sequence is closely similar to that reported for the product of gene A2-A3 of bacteriophage BF23. Segments of the sequence are similar to segments of bacteriophage T4 gene 32 protein , bacteriophage T3 RNA polymerase, and the protein encoded by the host gene responsible for isoenzyme conversion of alkaline phosphatase. The similarity of residues 26-46 to a portion of a rabbit lipopolysaccharide binding protein is possibly relevant to the function of the A2 protein.

Descriptors: *DNA-Binding Proteins; *T-Phages--genetics--GE; *Viral Proteins--isolation and purification--IP; Amino Acid Sequence; Endopeptidases; Escherichia coli--genetics--GE; Molecular Sequence Data; Peptide Fragments--isolation and purification--IP; Research Support, U.S. Gov't, P.H.S.; Software; Viral Proteins--chemistry--CH

CAS Registry No.: 0 (DNA-Binding Proteins); 0 (Peptide Fragments); 0 (Viral Proteins); 138898-68-7 (A2 protein, Bacteriophage T5)

Enzyme No.: EC 3.4.- (Endopeptidases)

Record Date Created: 19910731

Record Date Completed: 19910731

... bacteriophage BF23. Segments of the sequence are similar to segments of bacteriophage T4 gene 32 protein , bacteriophage T3 RNA polymerase, and the protein encoded by the host gene responsible for isoenzyme conversion of alkaline phosphatase. The similarity of residues 26-46 to a portion of a rabbit lipopolysaccharide binding protein is possibly relevant to the function of the A2 protein.

Chemical Name: DNA-Binding Proteins; Peptide Fragments; Viral Proteins; A2 protein , Bacteriophage T5; Endopeptidases

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$9.09 1.515 DialUnits File5
$6.60 3 Type(s) in Format 9
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$0.59 Estimated cost File35
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$0.45 Estimated cost File41
$0.86 0.172 DialUnits File45
$0.86 Estimated cost File45
$1.86 0.404 DialUnits File50
$1.86 Estimated cost File50
$0.40 0.097 DialUnits File65
$0.40 Estimated cost File65
$5.11 0.549 DialUnits File71
$2.25 1 Type(s) in Format 9
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$7.36 Estimated cost File71
$18.24 1.533 DialUnits File73
$26.40 8 Type(s) in Format 9
$26.40 8 Types
$44.64 Estimated cost File73
$0.23 0.054 DialUnits File91
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$0.42 0.100 DialUnits File98
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$0.29 Estimated cost File110
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$0.72 Estimated cost File135
$0.47 0.075 DialUnits File136
$0.47 Estimated cost File136
$0.24 0.081 DialUnits File143
$0.24 Estimated cost File143
$3.33 0.739 DialUnits File144

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          $1.65   1 Types
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     $0.72  Estimated cost File172
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     $0.63  Estimated cost File185
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     $0.00  Estimated cost File391
          $4.20     0.169 DialUnits File434
     $4.20  Estimated cost File434
          $0.25     0.039 DialUnits File467
     $0.25  Estimated cost File467
          OneSearch, 29 files, 10.494 DialUnits FileOS
     $2.40  TELNET
   $232.69  Estimated cost this search
   $232.72  Estimated total session cost  10.775 DialUnits

Logoff: level 05.17.01 D  09:05:26


You are now logged off
```